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DATE: Tuesday, April 09, 2002

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	DB=USPT,PGPB,JPAB,DWPI; PLUR=YES; OP=ADJ		
L9	('5985602'| '5962648'| '5959171'| '5905185'| '5892070'| '5756687'| '5750176'| '5741957'| '5567615')[ABPN1,NRPN,PN,WKU]	18	L9
L8	L6 and cellulose binding domain	1	L8
L7	L6 and protein L	1	L7
L6	L5 and matrix	177	L6
L5	L4 and purif$	324	L5
L4	l1 same l3	374	L4
L3	(protein$ or polypeptide$) near3 produc$	52232	L3
L2	(protein$ or polypeptide$) n3 production	0	L2
L1	transgen$ same milk	757	L1

END OF SEARCH HISTORY

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Web
NEWS 3 Jan 25 Searching with the P indicator for Preparations
NEWS 4 Jan 29 FSTA has been reloaded and moves to weekly updates
NEWS 5 Feb 01 DKILIT now produced by FIZ Karlsruhe and has a new update
         frequency
NEWS 6 Feb 19 Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 7 Mar 08 Gene Names now available in BIOSIS
NEWS 8 Mar 22 TOXLIT no longer available
NEWS 9 Mar 22 TRCTHERMO no longer available
NEWS 10 Mar 28 US Provisional Priorities searched with P in CA/CAplus
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=> e meade/au
E1        8   MEADDOUGH ERIKA/AU
E2        2   MEADDOUGH ERIKA L/AU
E3        1 --> MEADE/AU
E4       11   MEADE A/AU
E5        1   MEADE A B/AU
E6        3   MEADE A C/AU
E7        6   MEADE A G/AU
E8        5   MEADE A L/AU
E9        1   MEADE ABBY/AU
E10       1   MEADE ABIGAIL/AU
E11       3   MEADE ABIGAIL L/AU
E12       1   MEADE ADAMADIA DEFOREST AND BRUCE D/AU

=> s e3
L1        1 MEADE/AU

=> d bib abs

L1  ANSWER 1 OF 1  EMBASE  COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN  97334052 EMBASE
DN  1997334052
TI  Errata: 'Hormone replacement therapy and haemostatic function' in the
    State of the Art Book (Journal of the International Society on Thrombosis
    and Haemostasis (1997) 78(1)) (765-769)).
AU  ***Meade***
SO  Thrombosis and Haemostasis, (1997) 78/4 (1304).
    Refs: 0
    ISSN: 0340-6245  CODEN: THHADQ
CY  Germany
DT  Journal; Errata
FS  025   Hematology
LA  English


=> s meade h/au
L2       33 MEADE H/AU

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 33 ANSWERS - CONTINUE? Y/(N):y

L2  ANSWER 1 OF 33  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS
INC.
AN  2002:126881  BIOSIS
DN  PREV200200126881
TI  Transgenic production of antibodies in milk.
AU  ***Meade, H.*** ; Ditullio, P.; Pollock, D.
CS  Newton, Mass. USA
    ASSIGNEE: GENZYME TRANSGENICS CORPORATION
PI  US 5827690 Oct. 27, 1998
SO  Official Gazette of the United States Patent and Trademark Office Patents,
    (Oct. 27, 1998) Vol. 1215, No. 4, pp. 4074.
    ISSN: 0098-1133.
DT  Patent
LA  English

L2  ANSWER 2 OF 33  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS
INC.
AN  2002:109260  BIOSIS
DN  PREV200200109260
TI  Transgenic non-human mammal milk.
AU  ***Meade, H.*** ; Lonberg, N.
CS  Newton, Mass. USA
    ASSIGNEE: PHARMING B.V.
PI  US 5750172 May 12, 1998
SO  Official Gazette of the United States Patent and Trademark Office Patents,
    (May 12, 1998) Vol. 1210, No. 2, pp. 1447.
    ISSN: 0098-1133.
DT  Patent
LA  English

L2  ANSWER 3 OF 33  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS
INC.
AN  1999:142806  BIOSIS
DN  PREV199900142806
TI  The effect of DNA concentration microinjection on embryo survival, number
    of offspring born and transgenic rate in the caprine species.
AU  Gavin, W.; Pollock, D.; Wilburn, B.; Williams, J.; Melican, D.; Echelard,
    Y.; ***Meade, H.***
CS  Genzyme Transgenics Corporation, Framingham, MA 01701 USA
SO  Theriogenology, (Jan. 1, 1999) Vol. 51, No. 1, pp. 421.
    Meeting Info.: International Workshop on Embryogenesis and Implantation
    Kamuela, Hawaii, USA February 2-4, 1999
    ISSN: 0093-691X.
DT  Conference
LA  English

L2  ANSWER 4 OF 33  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS
INC.
AN  1999:135289  BIOSIS
DN  PREV199900135289
TI  Production of transgenic rabbits for the human glutamic acid
    decarboxylase.
AU  Yang, X. (1); Dai, Y.; Chen, L.; Tian, X. C. (1); ***Meade, H.*** ; Van
    De Velde, A. (1); Julian, M.; Reinhart, F.; Kaufman, D. L.; Ziomek, C.
CS  (1) Dep. Anim. Sci., Univ. Connecticut, Storrs, CT 06269-4040 USA
SO  Theriogenology, (Jan. 1, 1999) Vol. 51, No. 1, pp. 429.
    Meeting Info.: International Workshop on Embryogenesis and Implantation
    Kamuela, Hawaii, USA February 2-4, 1999
    ISSN: 0093-691X.
DT  Conference
LA  English

L2  ANSWER 5 OF 33  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS
INC.
AN  1999:75028  BIOSIS
DN  PREV199900075028
TI  Transgenic production of antibodies in milk.
AU  ***Meade, H.*** ; Ditullio, P.; Pollock, D.
CS  Newton, Mass. USA
    ASSIGNEE: GENZYME TRANSGENICS CORPORATION
PI  US 5849992 Dec. 15, 1998
```

SO Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 15, 1998) Vol. 1217, No. 3, pp. 2673.
ISSN: 0098-1133.
DT Patent
LA English

L2 ANSWER 6 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:69789 BIOSIS
DN PREV199900069789
TI Transgenically produced antithrombin III.
AU Ditullio, P.; ***Meade, H.*** ; Cole, E. S.
CS Framingham, Mass. USA
ASSIGNEE: GENZYME TRANSGENIC CORPORATION
PI US 5843705 Dec. 1, 1998
SO Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 1, 1998) Vol. 1217, No. 1, pp. 479.
ISSN: 0098-1133.
DT Patent
LA English

L2 ANSWER 7 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:9101 BIOSIS
DN PREV199900009101
TI Production of recombinant antibodies in the milk of transgenic animals.
AU Young, M. W. (1); ***Meade, H. (1)*** ; Curling, J. M.; Ziomek, C. A. (1); Harvey, M. (1)
CS (1) Genzyme Transgenics Corp., 5 Mountain Rd., Framingham, MA 01701-9322
USA
SO Research in Immunology, (July-Aug., 1998) Vol. 149, No. 6, pp. 609-610.
ISSN: 0923-2494.
DT Article
LA English

L2 ANSWER 8 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:146770 BIOSIS
DN PREV199800146770
TI Fish analysis of multiple transgene integration sites in a beta casein-antithrombin III goat line.
AU Williams, J. (1); Ponce De Leon, F. A.; Midura, P. (1); Harrington, M. (1); ***Meade, H. (1)*** ; Echelard, Y. (1)
CS (1) Genzyme Transgenics Corp., Framingham, MA 01701 USA
SO Theriogenology, (Jan. 1, 1998) Vol. 49, No. 1, pp. 398.
Meeting Info.: Annual Conference of the International Embryo Transfer Society Boston, Massachusetts, USA January 18-20, 1998
ISSN: 0093-691X.
DT Conference
LA English

L2 ANSWER 9 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:146769 BIOSIS
DN PREV199800146769
TI Analysis of factors affecting embryo transfers in the production of transgenic goats.
AU Wilburn, B.; Nims, S.; Cammuso, C.; Midura, P.; Oliver, A.; Smith, T. E.; Pollock, D.; ***Meade, H.*** ; Ziomek, C.; Echelard, Y.; Gavin, W. G.
CS Genzyme Transgenics Corp., Framingham, MA 01701-9322 USA
SO Theriogenology, (Jan. 1, 1998) Vol. 49, No. 1, pp. 397.
Meeting Info.: Annual Conference of the International Embryo Transfer Society Boston, Massachusetts, USA January 18-20, 1998
ISSN: 0093-691X.
DT Conference
LA English

L2 ANSWER 10 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1997:135553 BIOSIS
DN PREV199799434756
TI High-level expression of recombinant human prolactin in the milk of transgenic mice.
AU Wilburn, B. (1); Woodworth, L.; Gronbeck, A.; Lewis-Williams, J. (1); Harrington, M. (1); Pollock, D. (1); Richards, S. M.; ***Meade, H.*** *** (1)*** ; Echelard, Y. (1)
CS (1) Genzyme Transgenics Corp., One Mountain Road, Framingham, MA 01709 USA
SO Theriogenology, (1997) Vol. 47, No. 1, pp. 219.
Meeting Info.: Annual Conference of the International Embryo Transfer Society Nice, France January 12-14, 1997
ISSN: 0093-691X.
DT Conference; Abstract; Conference
LA English

L2 ANSWER 11 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1997:135548 BIOSIS
DN PREV199799434751
TI Expression of the antibody hBR96-2 in the milk of transgenic mice and production of hBR96-2 transgenic goats.
AU Gavin, W. G. (1); Pollock, D. (1); Fell, P.; Yelton, D.; Cammuso, C. (1); Harrington, M. (1); Lewis-Williams, J. (1); Midura, P. (1); Oliver, A. (1); Smith, T. E. (1); Wilburn, B. (1); Echelard, Y. (1); ***Meade, H.*** *** (1)***
CS (1) Genzyme Transgenics Corp., Framingham, MA 01701 USA
SO Theriogenology, (1997) Vol. 47, No. 1, pp. 214.
Meeting Info.: Annual Conference of the International Embryo Transfer Society Nice, France January 12-14, 1997
ISSN: 0093-691X.
DT Conference; Abstract; Conference
LA English

L2 ANSWER 12 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:331683 BIOSIS
DN PREV199699054039
TI Alterations of the physical characteristics of milk from transgenic mice producing bovine kappa-casein.
AU Gutierrez-Adan, A.; Maga, E. A.; ***Meade, H.*** ; Shoemaker, C. F.; Medrano, J. F.; Anderson, G. B.; Murray, J. D. (1)
CS (1) Dep. Animal Sci., Univ. Calif., Davis, CA 95616 USA
SO Journal of Dairy Science, (1996) Vol. 79, No. 5, pp. 791-799.
ISSN: 0022-0302.
DT Article
LA English
AB kappa-Casein is the protein fraction of milk that allows formation of micelles and determines micelle size and function, thus affecting many of the physical characteristics of milk. Several lines of transgenic mice were generated bearing the B allele of the bovine kappa-CN gene under the control of the regulatory sequences of the caprine beta-CN gene that specifically directed expression of bovine kappa-CN to the lactating mammary tissue of these mice. High expression of bovine kappa-CN protein was observed in the lines studied; the total level of protein in milk was not significantly affected. A high degree of conservation in the amino acids involved in the predicted three-dimensional structure exists between murine and bovine kappa-CN. Milk from transgenic lines expressing high bovine kappa-CN had a significantly smaller micelle size than did control milk. Therefore, bovine kappa-CN appears to have effectively participated in assembly of murine casein micelles. There was no effect on the time of rennet coagulation, but the association was significant between the milk of transgenic lines and the production of a stronger curd in rennet-induced gels. We conclude that bovine kappa-CN is an appropriate candidate for transgenic technology that would increase the ratio of kappa-CN to the calcium-sensitive caseins, therefore affecting the physical properties of the colloidal casein suspension.

L2 ANSWER 13 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:100857 BIOSIS
DN PREV199698672992
TI Effect of culture media on the development of microinjected murine embryos.
AU Wilburn, B.; Harvey, M.; Lewis-Williams, J.; Theodosiou, N.; Ditullio, P.; Liem, H.; Chen, L. H.; ***Meade, H.*** ; Echelard, Y.
CS Genzyme Transgenics Corp., Framingham, MA 01701 USA
SO Theriogenology, (1996) Vol. 45, No. 1, pp. 339.
Meeting Info.: Annual Conference of the International Embryo Transfer Society Salt Lake City, Utah, USA January 7-10, 1996
ISSN: 0093-691X.
DT Conference
LA English

L2 ANSWER 14 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:336310 BIOSIS
DN PREV199345031035
TI High level expression of tissue plasminogen activator using the goat beta-casein promoter.
AU Ditullo, P. (1); Pollock, J.; Roberts, B.; Vitale, J.; ***Meade, H.*** ; Ebert, K.
CS (1) Genzyme, Framington, MA 01701 USA
SO FASEB Journal, (1993) Vol. 7, No. 7, pp. A1223.
Meeting Info.: Joint Meeting of the American Society for Biochemistry and Molecular Biology and American Chemical Society Division of Biological Chemistry San Diego, California, USA May 30-June 3, 1993
ISSN: 0892-6638.
DT Conference
LA English

L2 ANSWER 15 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1990:388953 BIOSIS
DN BR39:59914
TI STRUCTURE-FUNCTION RELATIONSHIP OF CD4-PE HYBRID TOXINS.
AU WINKLER G; JAKUBOWSKI A; TURNER S; LIU T; HEANUE T; BURRUS B; MCGRAY P;
ROSA M; GRIFFFITHS B; THOMAS D; ***MEADE H***
CS BIOGEN, INC., CAMBRIDGE, MASS.
SO SIXTH INTERNATIONAL CONFERENCE ON AIDS. SIXTH INTERNATIONAL CONFERENCE ON
AIDS, VOLS. 1-3. PAGINATION VARIES SIXTH INTERNATIONAL CONFERENCE ON AIDS
UNIVERSITY OF CALIFORNIA SAN FRANCISCO: SAN FRANCISCO, CALIFORNIA, USA.

ILLUS. MAPS. PAPER. (1990) 0 (0), ABSTRACT THA 249.
DT Conference
FS BR; OLD
LA English

L2 ANSWER 16 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1990:294413 BIOSIS
DN BR39:12594
TI BOVINE ALPHA-S-1 CASEIN GENE SEQUENCES DIRECT HIGH LEVEL EXPRESSION OF
ACTIVE HUMAN UROKINASE IN MOUSE MILK.
AU ***MEADE H*** ; GATES L; LACY E; LONBERG N
CS GENPHARM INT., 2 EDWARDS COURT, BURLINGAME, CALIF. 94010.
SO Bio/Technology, (1990) 8 (5), 443-446.
CODEN: BTCHDA. ISSN: 0733-222X.
FS BR; OLD
LA English

L2 ANSWER 17 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1986:93242 BIOSIS
DN BA81:3658
TI CLONING OF ARG-G FROM STREPTOMYCES LOSS OF GENE IN ARG-NEGATIVE MUTANTS OF
STREPTOMYCES-CATTLEYA.
AU ***MEADE H***
CS FERMENTATION MICROBIOL. DEP., MERCK CO. INC., RAHWAY, N.J. 07065.
SO BIO-TECHNOLOGY (MARTINSVILLE), (1985) 3 (10), 917-918.
CODEN: BTCHDA. ISSN: 0733-222X.
FS BA; OLD
LA English
AB Genetic instability is common in commercial strains of Streptomyces. Streptomyces cattleya segregates Arg- mutants at high frequency. The argG gene from wild type S. cattleya was cloned by complementation of argG in Escherichia coli. DNA containing argG was used as a probe in Southern blot analysis of S. cattleya strains. Arg- mutants of S. cattleya have deleted the region of DNA encoding the argG gene.

L2 ANSWER 18 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1976:44572 BIOSIS
DN BR12:44572
TI CHROMOSOMAL MOBILIZATION IN RHIZOBIUM-MELILOTI.
AU ***MEADE H*** ; SIGNER E
SO Abstr. Annu. Meet. Am. Soc. Microbiol., (1976) 76, H54.
CODEN: ASMACK. ISSN: 0094-8519.
DT Conference
FS BR; OLD
LA Unavailable

L2 ANSWER 19 OF 33 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1973:102923 BIOSIS
DN BA55:2916
TI THE SITE OF ACTION OF INHIBITORS OF INITIATION OF PROTEIN SYNTHESIS IN
RETICULOCYTES.
AU BAGLIONI C; JACOBS-LORENA M; ***MEADE H***
SO BIOCHIM BIOPHYS ACTA, (1972) 277 (1), 188-197.
CODEN: BBACAQ. ISSN: 0006-3002.
FS BA; OLD
LA Unavailable

L2 ANSWER 20 OF 33 MEDLINE
AN 1999051090 MEDLINE
DN 99051090 PubMed ID: 9835426
TI Production of recombinant antibodies in the milk of transgenic animals.
AU Young M W; ***Meade H*** ; Curling J M; Ziomek C A; Harvey M
CS Genzyme Transgenics Corporation, Framingham, MA 01701-9322, USA.
SO RESEARCH IN IMMUNOLOGY, (1998 Jul-Aug) 149 (6) 609-10. Ref: 4
Journal code: R6E; 8907467. ISSN: 0923-2494.
CY France
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199901
ED Entered STN: 19990209
Last Updated on STN: 19990209
Entered Medline: 19990128

L2 ANSWER 21 OF 33 MEDLINE
AN 1998282199 MEDLINE
DN 98282199 PubMed ID: 9616152
TI Transgenically produced human antithrombin: structural and functional comparison to human plasma-derived antithrombin.
AU Edmunds T; Van Patten S M; Pollock J; Hanson E; Bernasconi R; Higgins E; Manavalan P; Ziomek C; ***Meade H*** ; McPherson J M; Cole E S
CS Cell and Protein Therapeutics Department, Genzyme Corp, and Genzyme Transgenics Corp, Framingham, MA 01701-9322, USA.
SO BLOOD, (1998 Jun 15) 91 (12) 4561-71.
Journal code: A8G; 7603509. ISSN: 0006-4971.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199806
ED Entered STN: 19980713
Last Updated on STN: 19980713
Entered Medline: 19980630
AB Recombinant human antithrombin (rhAT) produced in transgenic goat milk was
purified to greater than 99%. The specific activity of the rhAT was identical to human plasma-derived AT (phAT) in an in vitro thrombin inhibition assay. However, rhAT had a fourfold higher affinity for heparin than phAT. The rhAT was analyzed and compared with phAT by reverse phase high-performance liquid chromatography, circular dichroism, fluorophore-assisted carbohydrate electrophoresis (FACE), amino acid sequence, and liquid chromatography/mass spectrography peptide mapping. Based on these analyses, rhAT was determined to be structurally identical to phAT except for differences in glycosylation. Oligomannose structures were found on the Asn 155 site of the transgenic protein, whereas only complex structures were observed on the plasma protein. RhAT contained a GalNAc for galactose substitution on some N-linked oligosaccharides, as well as a high degree of fucosylation. RhAT was less sialylated than phAT and contained both N-acetylneuraminic and N-glycolylneuraminic acid. We postulate that the increase in affinity for heparin found with rhAT resulted from the presence of oligomannose-type structures on the Asn 155 glycosylation site and differences in sialylation.

L2 ANSWER 22 OF 33 MEDLINE
AN 1998108846 MEDLINE
DN 98108846 PubMed ID: 9447585
TI Urine as a substitute for milk?.
CM Comment on: Nat Biotechnol. 1998 Jan;16(1):75-9
AU ***Meade H*** ; Ziomek C
SO NATURE BIOTECHNOLOGY, (1998 Jan) 16 (1) 21-2.
Journal code: CQ3; 9604648. ISSN: 1087-0156.
CY United States
DT Commentary
News Announcement
LA English
FS Priority Journals
EM 199803
ED Entered STN: 19980326
Last Updated on STN: 19980326
Entered Medline: 19980313

L2 ANSWER 23 OF 33 MEDLINE
AN 96384390 MEDLINE
DN 96384390 PubMed ID: 8792278
TI Alterations of the physical characteristics of milk from transgenic mice producing bovine kappa-casein.
AU Gutierrez-Adan A; Maga E A; ***Meade H*** ; Shoemaker C F; Medrano J F; Anderson G B; Murray J D
CS University of California, Davis 95616, USA.
SO JOURNAL OF DAIRY SCIENCE, (1996 May) 79 (5) 791-9.
Journal code: HWV; 2985126R. ISSN: 0022-0302.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199611
ED Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961108
AB kappa-Casein is the protein fraction of milk that allows formation of micelles and determines micelle size and function, thus affecting many of the physical characteristics of milk. Several lines of transgenic mice were generated bearing the B allele of the bovine kappa-CN gene under the control of the regulatory sequences of the caprine beta-CN gene that specifically directed expression of bovine kappa-CN to the lactating mammary tissue of these mice. High expression of bovine kappa-CN protein was observed in the lines studied; the total level of protein in milk was not significantly affected. A high degree of conservation in the amino acids involved in the predicted three-dimensional structure exists between murine and bovine kappa-CN. Milk from transgenic lines expressing high bovine kappa-CN had a significantly smaller micelle size than did control milk. Therefore, bovine kappa-CN appears to have effectively participated in assembly of murine casein micelles. There was no effect on the time of rennet coagulation, but the association was significant between the milk of transgenic lines and the production of a stronger curd in rennet-induced gels. We conclude that bovine kappa-CN is an appropriate candidate for transgenic technology that would increase the ratio of kappa-CN to the calcium-sensitive caseins, therefore affecting the physical properties of the colloidal casein suspension.

L2 ANSWER 24 OF 33 MEDLINE
AN 90365980 MEDLINE
DN 90365980 PubMed ID: 1369989
TI Bovine alpha S1-casein gene sequences direct high level expression of active human urokinase in mouse milk.
AU ***Meade H*** ; Gates L; Lacy E; Lonberg N

CS Biogen Inc., Cambridge, MA 02142.
SO BIO/TECHNOLOGY, (1990 May) 8 (5) 443-6.
Journal code: AL1; 8309273. ISSN: 0733-222X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS B
EM 199010
ED Entered STN: 19950809
Last Updated on STN: 20000303
Entered Medline: 19901010
AB We have produced a line of transgenic mice carrying a hybrid bovine alpha S1 casein/human urokinase gene. Bovine alpha S1-casein gene regulatory sequences specifically direct expression of the human urokinase gene in lactating mammary tissue from these mice. Urokinase is a 54 kD protein with 9 disulfide bonds that is normally synthesized in the kidney; however, the casein/urokinase transgenic mice secrete active human urokinase into their milk at concentrations of 1-2 mg/ml. The mice show no other abnormalities. They give birth to, and nurse, normal sized healthy litters. Thus it is possible to produce high concentrations of a large, cysteine rich, non-milk protein in the milk of transgenic animals. This line of transgenic mice provides a model for the eventual production of transgenic farm animals producing high levels of recombinant proteins in their milk.

L2 ANSWER 25 OF 33 MEDLINE
AN 78145335 MEDLINE
DN 78145335 PubMed ID: 25105
TI Anti-allergy properties of PRD-92-Ea [5,5-dimethyl-11-oxo 5H, 11H-(2) benzopyrano (4,3-g) (1) benzopyran-9-carboxylic acid ethanolamine] [proceedings].
AU Beets J L; ***Meade H*** ; Morley J
SO BRITISH JOURNAL OF PHARMACOLOGY, (1978 Mar) 62 (3) 423P.
Journal code: B00; 7502536. ISSN: 0007-1188.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197806
ED Entered STN: 19900314
Last Updated on STN: 19950206
Entered Medline: 19780617

L2 ANSWER 26 OF 33 MEDLINE
AN 78039761 MEDLINE
DN 78039761 PubMed ID: 921703
TI Genetic mapping of Rhizobium meliloti using RP4.
AU ***Meade H***
SO BASIC LIFE SCIENCES, (1977) 9 91-4.
Journal code: 9K0; 0360077. ISSN: 0090-5542.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197712
ED Entered STN: 19900314
Last Updated on STN: 19900314
Entered Medline: 19771229

L2 ANSWER 27 OF 33 MEDLINE
AN 72261774 MEDLINE
DN 72261774 PubMed ID: 5053770
TI The site of action of inhibitors of initiation of protein synthesis in reticulocytes.
AU Baglioni C; Jacobs-Lorena M; ***Meade H***
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1972 Aug 16) 277 (1) 188-97.
Journal code: A0W; 0217513. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197210
ED Entered STN: 19900310
Last Updated on STN: 19970203
Entered Medline: 19721012

L2 ANSWER 28 OF 33 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2002026056 EMBASE
TI A recombinant vaccine expressed in the milk of transgenic mice protects Aotus monkeys from a lethal challenge with Plasmodium falciparum.
AU Stowers A.W.; Chen L.-H.; Zhang Y.; Kennedy M.C.; Zou L.; Lambert L.; Rice T.J.; Kaslow D.C.; Saul A.; Long C.A.; ***Meade H.*** ; Miller L.H.
CS A.W. Stowers, Malaria Vaccine Development Unit, Laboratory of Parasitic Diseases, Natl. Inst. Allergy/Infectious Dis., 5640 Fishers Lane, Rockville, MD 20852, United States. astowers@niaid.nih.gov
SO Proceedings of the National Academy of Sciences of the United States of America, (8 Jan 2002) 99/1 (339-344).
Refs: 23
ISSN: 0027-8424 CODEN: PNASA6
CY United States
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB Two strains of transgenic mice have been generated that secrete into their milk a malaria vaccine candidate, the 42-kDa C-terminal portion of Plasmodium falciparum merozoite surface protein 1 (MSP1(42)). One strain secretes an MSP1(42) with an amino acid sequence homologous to that of the FVO parasite line, the other an MSP1(42) where two putative N-linked glycosylation sites in the FVO sequence have been removed. Both forms of MSP1(42) were purified from whole milk to greater than 91% homogeneity at high yields. Both proteins are recognized by a panel of monoclonal antibodies and have identical N termini, but are clearly distinguishable by some biochemical properties. These two antigens were each emulsified with Freund's adjuvant and used to vaccinate Aotus nancymai monkeys, before challenge with the homologous P. falciparum FVO parasite line. Vaccination with a positive control molecule, a glycosylated form of MSP1(42) produced in the baculovirus expression system, successfully protected five of six monkeys. By contrast, vaccination with the glycosylated version of milk-derived MSP1(42) conferred no protection compared with an adjuvant control. Vaccination with the nonglycosylated, milk-derived MSP1(42) successfully protected the monkeys, with 4/5 animals able to control an otherwise lethal infection with P. falciparum compared with 1/7 control animals. Analysis of the different vaccines used suggested that the differing nature of the glycosylation patterns may have played a critical role in determining efficacy. This study demonstrates the potential for producing efficacious malarial vaccines in transgenic animals.

L2 ANSWER 29 OF 33 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1999361820 EMBASE
TI Production of recombinant antibodies in the milk of transgenic animals.
AU Young M.W.; ***Meade H.*** ; Curling J.M.; Ziomek C.A.; Harvey M.; Falkenberg F.W.; Reddington J.J.; Peterson N.; Lipman N.S.; Jackson L.R.; Shi Y.; Marx U.; Pennell C.A.
CS M.W. Young, Genzyme Transgenics Corporation, 5 Mountain Road, Framingham, MA 01701-9322, United States
SO Research in Immunology, (1998) 149/6 (609-620).
Refs: 4
ISSN: 0923-2494 CODEN: RIMME5
CY France
DT Journal; (Short Survey)
FS 029 Clinical Biochemistry
037 Drug Literature Index
LA English

L2 ANSWER 30 OF 33 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1998195872 EMBASE
TI Transgenically produced human antithrombin: Structural and functional comparison to human plasma-derived antithrombin.
AU Edmunds T.; Van Patten S.M.; Pollock J.; Hanson E.; Bernasconi R.; Higgins E.; Manavalan P.; Ziomek C.; ***Meade H.*** ; McPherson J.M.; Cole E.S.
CS Dr. E.S. Cole, Cell/Protein Therapeutics Department, Genzyme Corp, 1 Mountain Rd, Framingham, MA 01701-9322, United States
SO Blood, (1998) 91/12 (4561-4571).
Refs: 60
ISSN: 0006-4971 CODEN: BLOOAW
CY United States
DT Journal; Article
FS 025 Hematology
029 Clinical Biochemistry
LA English
SL English
AB Recombinant human antithrombin (rhAT) produced in transgenic goat milk was purified to greater than 99%. The specific activity of the rhAT was identical to human plasma-derived AT (phAT) in an in vitro thrombin inhibition assay. However, thAT had a fourfold higher affinity for heparin than phAT. The rhAT was analyzed and compared with phAT by reverse phase high-performance liquid chromatography, circular dichroism, fluorophore-assisted carbohydrate electrophoresis (FACE), amino acid sequence, and liquid chromatography/mass spectrography peptide mapping. Based on these analyses, rhAT was determined to be structurally identical to phAT except for differences in glycosylation. Oligomannose structures were found on the Asn 155 site of the transgenic protein, whereas only complex structures were observed on the plasma protein. RhAT contained a GalNAc for galactose substitution on some N-linked oligosaccharides, as well as a high degree of fucosylation. RhAT was less sialylated than phAT and contained both N- acetylneuraminic and N-glycolylneuraminic acid. We postulate that the increase in affinity for heparin found with rhAT resulted from the presence of oligomannose-type structures on the Asn 155 glycosylation site and differences in sialylation.

L2 ANSWER 31 OF 33 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 90151766 EMBASE
DN 1990151766
TI Bovine alpha(S1)-casein gene sequences direct high level expression of active human urokinase in mouse milk.
AU ***Meade H.*** ; Gates L.; Lacy E.; Lonberg N.
CS Biogen Inc., 14 Cambridge Center, Cambridge MA 02142, United States
SO Bio/Technology, (1990) 8/5 (443-446).
ISSN: 0733-222X CODEN: BTCHDA
CY United States

DT Journal; Article
FS 004 Microbiology
022 Human Genetics
029 Clinical Biochemistry
LA English
SL English
AB We have produced a line of transgenic mice carrying a hybrid bovine .alpha.(S1) casein/human urokinase gene. Bovine .alpha.(S1)-casein gene regulatory sequences specifically direct expression of the human urokinase gene in lactating mammary tissue from these mice. Urokinase is a 54 kD protein with 9 disulfide bonds that is normally synthesized in the kidney; however, the casein/urokinase transgenic mice secrete active human urokinase into their milk at concentrations of 1-2 mg/ml. The mice show no other abnormalities. They give birth to, and nurse, normal sized healthy litters. Thus it is possible to produce high concentrations of a large, cysteine rich, non-milk protein in the milk of transgenic animals. This line of transgenic mice provides a model for the eventual production of transgenic farm animals producing high levels of recombinant proteins in their milk.

L2 ANSWER 32 OF 33 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 87056708 EMBASE
DN 1987056708
TI Diabetes and injection site infections in children.
AU Stutchfield P.R.; ***Meade H.*** ; Brown B.; et al.
CS Royal Liverpool Children's Hospital (Alder Hey), Liverpool L12 2AP, United Kingdom
SO Practical Diabetes, (1986) 3/6 (298-300).
CODEN: PRDIEN
CY United Kingdom
DT Journal
FS 003 Endocrinology
007 Pediatrics and Pediatric Surgery
004 Microbiology
013 Dermatology and Venereology
LA English
AB Injection site infections in a paediatric diabetic population are reviewed and possible aetiological factors examined. The incidence of infection was found to be significantly higher (5%) than that reported from adult clinics. Adolescent girls are particularly at risk.

L2 ANSWER 33 OF 33 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 78296153 EMBASE
DN 1978296153
TI Anti-allergy properties of PRD-92-Ea [5,5-dimethyl-11-oxo 5H,11H-(2) benzopyrano (4,3-g) (1) benzopyran-9-carboxylic acid ethanolamine].
AU Beets J.L.; ***Meade H.*** ; Morley J.
CS Dept. Clin. Pharmacol., Cardiothorac. Inst. Brompton Hosp., London, United Kingdom
SO British Journal of Pharmacology, (1978) 62/3 (423P).
CODEN: BJPCBM
CY United Kingdom
DT Journal
FS 037 Drug Literature Index
LA English

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L3 26 DUP REM L2 (7 DUPLICATES REMOVED)

=> s protein? or polypeptide?
L4 4002780 PROTEIN? OR POLYPEPTIDE?

=> s l4 (3a) purif?
L5 107841 L4 (3A) PURIF?

=> s l5 and transgen?
L6 495 L5 AND TRANSGEN?

=> s l4 (3a) milk
L7 20753 L4 (3A) MILK

=> s l7 (3a) purif?
L8 160 L7 (3A) PURIF?

=> s l8 and transgen?
L9 26 L8 AND TRANSGEN?

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L10 15 DUP REM L9 (11 DUPLICATES REMOVED)

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L10 ANSWER 1 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2001:228212 BIOSIS
DN PREV200100228212
TI Lysosomal proteins produced in the milk of ***transgenic*** animals.
AU Reuser, Arnold J. J. (1); Van der Ploeg, Ans T.; Pieper, Frank R.; Verbeet, Martin Ph.
CS (1) Rotterdam Netherlands
ASSIGNEE: Pharming B.V., Leiden, Netherlands; The Universiteit Leiden, Leiden, Netherlands; Academic Hospital, Rotterdam, Netherlands; Eramus Universiteit, Rotterdam, Netherlands
PI US 6118045 September 12, 2000
SO Official Gazette of the United States Patent and Trademark Office Patents, (Sep. 12, 2000) Vol. 1238, No. 2, pp. No Pagination. e-file.
ISSN: 0098-1133.
DT Patent
LA English
AB The invention provides ***transgenic*** nonhuman mammals producing phosphorylated lysosomal proteins in their milk, and methods of generating the same. Phosphorylation occurs at the 6' position of a mannose side chain residue. Also provided are methods of ***purifying*** lysosomal ***proteins*** from ***milk*** , and incorporating the ***proteins*** into pharmaceutical compositions for use in enzyme replacement therapy.

L10 ANSWER 2 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
1
AN 2000:190930 BIOSIS
DN PREV200000190930
TI High expression of the human hepatocarcinoma-intestine-pancreas/pancreatic-associated protein (HIP/PAP) gene in the mammary gland of lactating ***transgenic*** mice: Secretion into the milk and purification of the HIP/PAP lectin.
AU Christa, Laurence (1); Pauloin, Alain; Simon, Marie-Therese; Stinnakre, Marie-Georges; Fontaine, Marie-Louise; Delpal, Serge; Ollivier-Bousquet, Michele; Brechot, Christian; Devinoy, Eve
CS (1) Institut National de la Sante et de la Recherche Medicale U-370 and Liver Unit, Centre Hospitalier Universitaire Necker, 156 rue de Vaugirard, 75742, Paris cedex, 15 France
SO European Journal of Biochemistry, (March, 2000) Vol. 267, No. 6, pp. 1665-1671.
ISSN: 0014-2956.
DT Article
LA English
SL English
AB The human hepatocarcinoma-intestine-pancreas/pancreatic-associated protein
(HIP/PAP) gene was previously identified because of its increased expression in primary liver cancers and during the acute phase of pancreatitis. In normal tissues, HIP/PAP is expressed both in endocrine and exocrine cells of the intestine and pancreas. HIP/PAP is a lactose binding C-type lectin which acts as an adhesion molecule for rat hepatocytes. The aim of the work was to study the HIP/PAP secretory pathway and to produce high levels of HIP/PAP in the milk of lactating ***transgenic*** mice. In view of its lactose C-type lectin properties, we have studied the consequences of the expression of HIP/PAP on mammary epithelial cells. In homozygous mice, production reached 11.2 mgcntdotmL-1 of milk. High levels of soluble and pure HIP/PAP (18.6 mg) were purified

from 29 mL of ***milk*** . The ***purified*** ***protein*** was sequenced and the N-terminal amino acid of the mature HIP/PAP was identified as Glu27, thus localizing the site of cleavage of the signal peptide. The HIP/PAP ***transgene*** was only expressed in the mammary gland of lactating ***transgenic*** mice. HIP/PAP was detected by immunofluorescence in the whole gland, but labelling was heterogeneous between alveolar clusters, with strongly positive sparse cells. Using immuno electron microscopy, HIP/PAP was observed in all the compartments of the secretory pathway within the mammary epithelial cells. We provide evidence that HIP/PAP is secreted through the Golgi pathway. However, the number of distended Golgi saccules was increased when compared to that found in wild-type mouse mammary cells. These modifications could be related to HIP/PAP C-type lectin specific properties.

L10 ANSWER 3 OF 15 MEDLINE
AN 2000504294 MEDLINE
DN 20506029 PubMed ID: 11051810
TI Production of pharmaceutical proteins with mammary gland bioreactor.
AU Liu S; Liang G D
CS National Laboratory of Molecular Virology and Genetic Engineering, Institute of Virology, Beijing.
SO SHENG WU KUNG CH ENG HSUEH PAO, (2000 Jul) 16 (4) 421-4.
Journal code: DJD. ISSN: 1000-3061.
CY China
DT Journal; Article; (JOURNAL ARTICLE)
LA Chinese
FS Priority Journals
EM 200011
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001113
AB Mammary gland bioreactor is a useful biological system which expresses foreign genes in the mammary gland and produces functional pharmaceutical proteins in milk. This production route is appealing for it's advantages, such as the simplicity of access to the expressed protein, the high production of the mammary gland, the capabilities to perform translational modifications. As an alternative of cell culture systems, it is a new biotechnology. The article reviews some aspects on generation and characterization of mammary gland bioreactor, separation and ***purification*** of foreign ***protein*** from ***milk*** and some questions that need to be answered on the route.

L10 ANSWER 4 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
2
AN 2001:48661 BIOSIS
DN PREV200100048661
TI ***Transgenic*** animal bioreactors.
AU Houdebine, Louis Marie (1)
CS (1) Unite de Biologie du Developpement et Biotechnologie, Institut National de la Recherche Agronomique, 78352, Jouy-en-Josas Cedex: houdebine@biotec.jouy.inra.fr France
SO Transgenic Research, (2000) Vol. 9, No. 4-5, pp. 305-320. print.
ISSN: 0962-8819.
DT Article
LA English
SL English
AB The production of recombinant proteins is one of the major successes of biotechnology. Animal cells are required to synthesize proteins with the appropriate post-translational modifications. ***Transgenic*** animals are being used for this purpose. Milk, egg white, blood, urine, seminal plasma and silk worm cocoon from ***transgenic*** animals are candidates to be the source of recombinant proteins at an industrial scale. Although the first recombinant protein produced by ***transgenic*** animals is expected to be in the market in 2000, a certain number of technical problems remain to be solved before the various systems are optimized. Although the generation of ***transgenic*** farm animals has become recently easier mainly with the technique of animal cloning using transfected somatic cells as nuclear donor, this point remains a limitations as far as cost is concerned. Numerous experiments carried out for the last 15 years have shown that the expression of the ***transgene*** is predictable only to a limited extent. This is clearly due to the fact that the expression vectors are not constructed in an appropriate manner. This undoubtedly comes from the fact that all the signals contained in genes have not yet been identified. Gene constructions thus result sometime in poorly functional expression vectors. One possibility consists in using long genomic DNA fragments contained in YAC or BAC vectors. The other relies on the identification of the major important elements required to obtain a satisfactory ***transgene*** expression. These elements include essentially gene insulators, chromatin openers, matrix attached regions, enhancers and introns. A certain number of proteins having complex structures (formed by several subunits, being glycosylated, cleaved carboxylated...) have been obtained at levels sufficient for an industrial exploitation. In other cases, the mammary cellular machinery seems insufficient to promote all the post-translational modifications. The addition of genes coding for enzymes involved in protein maturation has been envisaged and successfully performed in one case. Furin gene expressed specifically in the mammary gland proved to able to cleave native human protein C with good efficiency. In a certain number of cases, the recombinant proteins produced in milk have deleterious effects on the mammary gland function or in the animals themselves. This comes independently from ectopic expression of the ***transgenes*** and from the transfer of the recombinant proteins from milk to blood. One possibility to eliminate or reduce these side-effects may be to use systems inducible by an exogenous molecule such as tetracycline allowing the ***transgene*** to be expressed only during lactation and strictly in the mammary gland. The ***purification*** of recombinant ***proteins*** from ***milk*** is generally not particularly difficult. This may not be the case, however, when the endogenous proteins such as serum albumin or antibodies are abundantly present in milk. This problem may be still more crucial if proteins are produced in blood. Among the biological contaminants potentially present in the recombinant proteins prepared from ***transgenic*** animals, prions are certainly those raising the major concern. The selection of animals chosen to generate ***transgenic*** on one hand and the elimination of the potentially contaminated animals, thanks to recently defined quite sensitive tests may reduce the risk to an extremely low level. The available techniques to produce pharmaceutical proteins in milk can be used as well to optimize milk composition of farm animals, to add nutriceuticals in milk and potentially to reduce or even eliminate some mammary infectious diseases.

L10 ANSWER 5 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
3
AN 1998:205689 BIOSIS
DN PREV199800205689
TI Zn2+-selective ***purification*** of recombinant ***proteins*** from the ***milk*** of ***transgenic*** animals.
AU Degener, Arthur; Belew, Makonnen; Velander, William H. (1)
CS (1) Dep. Chem. Engineering, Va. Polytechnic Inst. and State Univ., Blacksburg, VA USA
SO Journal of Chromatography A, (March 13, 1998) Vol. 799, No. 1-2, pp. 125-137.
ISSN: 0021-9673.
DT Article
LA English
AB The milk of ***transgenic*** livestock is becoming a viable, large-scale source of post-translationally complex, recombinant therapeutic proteins. Recombinant vitamin K-dependent proteins such as human protein C (rhPC) and Factor IX can be produced in milk. However, rate limitations in post-translational modification such as intrachain proteolytic cleavage and gamma-carboxylation occur in the mammary gland. Thus, most desirable recombinant products often exist as sub-populations in milk because the mammary gland tends to secrete incompletely processed polypeptides. In general, a nonaffinity purification strategy by which to ***purify*** mature recombinant ***proteins*** from ***milk*** is desirable. Zn2+ is used to selectively modify ion-exchange adsorption behavior of endogenous and recombinant milk proteins through conformational changes which cause aggregation and or precipitation. Zn2+-selective precipitation of ***milk*** and recombinant ***proteins*** results in the ***purification*** of active rhPC at high yield from the milk of ***transgenic*** pigs using expanded bed chromatography. This method selects for rhPC which is both heterodimeric and properly gamma-carboxylated. Due to the homology of milk proteins among different species, this same Zn2+-selective precipitation strategy is useful for developing purification methods for other recombinant proteins from the milk of ***transgenic*** livestock.

L10 ANSWER 6 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:495790 BIOSIS
DN PREV199800495790
TI ***Transgenics*** and dairy animal reproduction: Current status and potential.
AU Keefer, Carol L. (1)
CS (1) Nexia Biotechnol. Inc., Ste. Anne de Bellevue, PQ H9X 3V9 Canada
SO Bovine Practitioner, (Jan., 1998) Vol. 0, No. 32 PART 1, pp. 63-67.
ISSN: 0524-1685.
DT Article
LA English
AB Application of ***transgenic*** technology to domestic animals has been limited in the past. Improvements in reproductive techniques, including in vitro embryo production, and economic incentives have lead to the implementation of ***transgenic*** programs by commercial groups. ***Transgenic*** technology incorporates molecular and reproductive techniques in order to direct and harness the tremendous protein synthetic capacity of the mammary gland of dairy animals. ***Transgenic*** animals (animals which have exogenous DNA stably integrated into their genome) can be used to express value-added exogenous ***proteins*** in their ***milk*** for subsequent ***purification*** or to increase ***milk*** ***protein*** and calcium concentration in their milk for increased efficiency of production of processed dairy foods.

L10 ANSWER 7 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:75958 BIOSIS
DN PREV199800075958
TI Characterization and partial purification of bovine alpha-lactalbumin and beta-casein produced in milk of ***transgenic*** mice.
AU Jeng, Shin-Yi; Bleck, Gregory T.; Wheller, Matthew B.; Jimenez-Flores, Rafael (1)
CS (1) Dep. Dairy Sci., Calif. Polytechnic State Univ., San Luis, Obsipo 93407, CA USA
SO Journal of Dairy Science, (Dec., 1997) Vol. 80, No. 12, pp. 3167-3175.
ISSN: 0022-0302.

DT Article
LA English
AB Bovine alpha-lactalbumin (alpha-LA) and bovine beta-casein (beta-CN), from milk from ***transgenic*** mice were characterized and partially purified using electrophoretic, immunoblotting, and chromatographic methods. The ***transgenically*** expressed bovine milk proteins were identified using PAGE or by a combination of preparative isoelectrofocusing followed by Western immunoblotting. The heterologous bovine alpha-1A and bovine beta-CN had molecular masses that were identical to those of the native proteins and pI values that were similar to those of the native proteins. The estimated expression of the proteins was 1.0 mg/ml of milk for alpha-LA and 3.0 mg/ml for beta-CN. The calcium binding of bovine alpha-LA suggested that the protein produced in murine milk has the same electrophoretic shift as native bovine alpha-LA after the removal of calcium. Nitrogen-linked glycosylation of native and murine synthesized bovine alpha-LA was identified by peptide-N-glycosidase F treatment, and the N-terminal amino acid sequence of HPLC-purified bovine alpha-LA from mouse milk was confirmed to be identical to native bovine alpha-LA. In addition, the phosphorylation of the bovine beta-CN expressed in the milk of ***transgenic*** mice was the same as that of native bovine beta-CN, as determined by phosphatase digestion.

L10 ANSWER 8 OF 15 MEDLINE DUPLICATE 4
AN 97414792 MEDLINE
DN 97414792 PubMed ID: 9269458
TI Separation of recombinant human protein C from ***transgenic*** animal milk using immobilized metal affinity chromatography.
AU Dalton J C; Bruley D F; Kang K A; Drohan W N
CS Department of Chemical and Biochemical Engineering, University of Maryland, Baltimore County (UMBC) 21228, USA.
SO ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, (1997) 411 419-28.
Journal code: 2LU; 0121103. ISSN: 0065-2598.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199710
ED Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971020
AB Protein C is an important serine protease due to its ability to proteolytically cleave activated Factors V and VIII. Excess coagulation and blood agglutination can lead to plugged capillaries, thereby reducing oxygen transport to interstitial tissues. To treat patients with hereditary and acquired protein C deficiency would require a greater amount of Protein C than that available from human plasma. However, the potential demand for this protein could be met by the production of human protein C from ***transgenic*** animal mammary glands. Thus, research into inexpensive, efficient methods to ***purify*** ***proteins*** from ***transgenic*** animal ***milk*** will be a critical area of study for the large scale production of protein C. Immobilized metal affinity chromatography (IMAC) is a novel method for the purification of protein C. A proposed method of purification is to take advantage of protein C's strong metal ion binding characteristics with IMAC to assist in the separation from ***transgenic*** animal milk. The separation procedure is benchmarked against current systems in use by the American Red Cross for purification of Protein C from ***transgenic*** porcine milk. Common problems in developing separation schemes for new therapeutics are the initial availability of the product (protein), and time-to-market concerns. Extensive experimental tests for scaleable purification schemes are often cost and time prohibitive. In order to optimize an IMAC protocol with minimal waste of time and resources, total quality management tools have been adopted. Initial experiments were designed to choose buffer conditions, eluents, immobilized valence metals, and flow rates using Taguchi experimental design, which is a total quality management (TQM) tool. One of the values of Taguchi methods lies in the use of Latin orthogonal sets. Through the use of the orthogonal sets, the total number of experiments may be reduced, shortening the focus time on optimal conditions.

L10 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
5
AN 1997:482131 BIOSIS
DN PREV199799781334
TI Aqueous two-phase partitioning of milk proteins: Application to human protein C secreted in pig milk.
AU Cole, Kenneth D. (1); Lee, Timothy K.; Lubon, Henrik
CS (1) Biotechnol. Div., Natl. Inst. Standards Technol., Gaithersburg, MD 20899 USA
SO Applied Biochemistry and Biotechnology, (1997) Vol. 67, No. 1-2, pp. 97-112.
ISSN: 0273-2289.
DT Article
LA English
AB Milk of ***transgenic*** pigs secreting recombinant human Protein C (rHPC) was used as a model system to determine the utility of aqueous two-phase extraction systems (ATPS) for the initial step in the ***purification*** of ***proteins*** from ***milk*** . The major challenges in ***purification*** of recombinant ***proteins*** from ***milk*** are removal of casein micelles (that foul processing equipment) and elimination of the host milk proteins from the final product. When milk was partitioned in ATPS composed of polyethylene glycol (PEG) and ammonium sulfate (AS), the phases were clarified and most of the caseins precipitated at the interphase. The partition coefficients of the major milk proteins and rHPC were dependent upon the molecular weight of the PEG used in the ATPS. Higher-partition coefficients of the major whey proteins, beta-lactoglobulin, and alpha-lactalbumin were observed in ATPS made up of lower molecular-weight PEG (1000 or 1450) as compared to systems using higher molecular-weight PEG. Lowering the pH of the ATPS from 7.5 to 6.0 resulted in increased precipitation of the caseins and decreased their concentration in both phases. rHPC had a partition coefficient of 0.04 in a system composed of AS and PEG 1450. The rHPC in pig milk was shown to be highly heterogenous by two-dimensional gel electrophoresis. The heterogeneity was owing to inefficient proteolytic processing of the single chain to the heterodimeric form and differences in glycosylation and other post-translational processing. Differential partitioning of the multiple forms of purified rHPC in the ATPS was not observed. rHPC after processing in ATPS was recovered in a clear phase free of most major milk proteins. ATPS are useful as the initial processing step in the ***purification*** of recombinant ***proteins*** from ***milk*** because clarification and enrichment is combined in a single step.

L10 ANSWER 10 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
6
AN 1998:297228 BIOSIS
DN PREV199800297228
TI The application of aqueous two-phase systems to the ***purification*** of pharmaceutical ***proteins*** from ***transgenic*** sheep ***milk*** .
AU Harris, D. P. (1); Andrews, A. T.; Wright, G.; Pyle, D. L.; Asenjo, J. A.
CS (1) Food Science Dep., New Zealand Dairy Research Institute, Private Bag 11029, Palmerston North New Zealand
SO Bioseparation, (1997) Vol. 7, No. 1, pp. 31-37.
ISSN: 0923-179X.
DT Article
LA English
AB ***Transgenic*** sheep milk containing the protein human alpha1-Antitrypsin (AAT) was partitioned in Poly(ethylene glycol) (PEG)-Sulphate and PEG-Phosphate biphasic systems. Individual partition coefficients for AAT and some of the milk proteins were determined in these systems. The effects of PEG molecular weight, pH and the inclusion of NaCl on the partitioning of the proteins were also studied. It was found that increasing the concentration of NaCl and decreasing the molecular weight of the PEG resulted in an increase of the partition coefficients of the proteins to the upper (PEG) phase. This partitioning effect was greater for the more hydrophobic proteins and particularly in systems having a pH close to the isoelectric point of the protein. Solubilities of the proteins in increasing concentrations of ammonium sulphate were measured in order to investigate the effects of hydrophobic and electrostatic interactions on the partitioning of these proteins in aqueous two-phase systems. Those proteins that precipitated at low levels of ammonium sulphate showed an increase in partition coefficient at low concentrations of NaCl, or they were precipitated at the interface of the phases at low concentrations of NaCl. Proteins that had low salting out constants in ammonium sulphate solutions were relatively unaffected by NaCl in ATPS. It is probable however that conformational changes and the state of aggregation of proteins are also important and. should be invoked in describing the partitioning behavior observed for beta-Lg for example. Comparison of theoretical and experimental values for AAT yield and purity showed clearly that partition coefficients are influenced by the degree of purity and values obtained with purified standards are not necessarily the same as for the same protein present in a complex mixture. Under the most favourable conditions using a 4% w/w loading of ***transgenic*** ovine milk, we obtained a 91% yield of AAT in the PEG phase with a purity of 73%.

L10 ANSWER 11 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
7
AN 1996:122992 BIOSIS
DN PREV199698695127
TI Synthesis and secretion of the mouse whey acidic protein in ***transgenic*** sheep.
AU Wall, Robert J. (1); Rexroad., Caird E., Jr.; Powell, Anne; Shamay, Avi; McKnight, Robert; Hennighausen, Lothar
CS (1) Gene Evaluation Mapping Lab., Agric. Res. Serv., USDA, Beltsville, MD 20705 USA
SO Transgenic Research, (1996) Vol. 5, No. 1, pp. 67-72.
ISSN: 0962-8819.
DT Article
LA English
AB The synthesis of foreign proteins can be targeted to the mammary gland of ***transgenic*** animals, thus permitting commercial ***purification*** of otherwise unavailable ***proteins*** from ***milk*** . Genetic regulatory elements from the mouse whey acidic protein (WAP) gene have been used successfully to direct expression of ***transgenes*** to the mammary gland of mice, goats and pigs. To extend the practical usefulness of WAP promoter-driven fusion genes and further characterize WAP expression in heterologous species, we introduced a 6.8 kb DNA fragment containing the genomic form of the mouse WAP gene into sheep zygotes. Two lines of ***transgenic*** sheep were produced. The ***transgene*** was expressed in mammary tissue of both lines and intact

WAP was secreted into milk at concentrations estimated to range from 100 to 500 mg/litre. Ectopic WAP gene expression was found in salivary gland, spleen, liver, lung, heart muscle, kidney and bone marrow of one founder ewe. WAP RNA was not detected in skeletal muscle and intestine. These data suggest that unlike pigs, sheep may possess nuclear factors in a variety of tissues that interact with WAP regulatory sequences. Though the data presented are based on only two lines, these findings suggest WAP regulatory sequences may not be suitable as control elements for ***transgenes*** in sheep bioreactors.

L10 ANSWER 12 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:253195 BIOSIS
DN PREV199698809324
TI Expanded bed ***purification*** of a recombinant ***protein*** from the ***milk*** of ***transgenic*** livestock.
AU Degener, Arthur (1); Belew, Makonnen; Velander, William H. (1)
CS (1) Dep. Chem. Eng., Va. Polytech. Inst. State Univ., Blacksburg, VA USA
SO Abstracts of Papers American Chemical Society, (1996) Vol. 211, No. 1-2, pp. BIOT 87.
Meeting Info.: 211th American Chemical Society National Meeting New Orleans, Louisiana, USA March 24-28, 1996
ISSN: 0065-7727.
DT Conference
LA English

L10 ANSWER 13 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:200390 BIOSIS
DN PREV199344096640
TI ***Purification*** of ***protein*** from ***milk*** of ***transgenic*** animals.
AU Wilkins, Tracy D.
CS Va. Tech Transpharm. Inc., Blacksburg, VA USA
SO Journal of Cellular Biochemistry Supplement, (1993) Vol. 0, No. 17 PART A, pp. 39.
Meeting Info.: Keystone Symposium on Protein Purification and Biochemical Engineering Santa Fe, New Mexico, USA January 15-21, 1993
ISSN: 0733-1959.
DT Conference
LA English

L10 ANSWER 14 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
8
AN 1992:475720 BIOSIS
DN BA94:107095
TI ISOLATION OF RECOMBINANT PROTEINS FROM MILK.
AU WILKINS T D; VELANDER W
CS TRANSPHARM/TECHLAB INC., BLACKSBURG, VA. 24061.
SO J CELL BIOCHEM, (1992) 49 (4), 333-338.
CODEN: JCEBD5. ISSN: 0730-2312.
FS BA; OLD
LA English
AB Milk is a complex bio-colloid which presents some unique problems for the protein isolation chemist, but the majority of the processing criteria for purifying recombinant proteins are the same as with any complex biological mixture. The casein micelles and fat globules behave as separate phases; they prevent filtration of the milk and interfere with the usual separation methods. The usual first step is to centrifuge the milk to remove the fat and precipitate the casein micelles with low pH or precipitating agents. Some recombinant proteins may associate to some degree with the micelles which may necessitate solubilizing them with chelating agents. If the majority of the product protein associates with either the fat or micelles, this can be used to advantage. Once the casein micelles have been removed or disrupted, the clarified milk can be processed by the usual separation methods. There also are proteases in milk which can degrade recombinant proteins. The greatest advantage of producing recombinant proteins in milk is the high concentration which can be obtained. The high levels of product protein can alleviate many problems associated with the application of classical ***purification*** strategies to ***transgenic*** ***milk*** ***proteins*** .

L10 ANSWER 15 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:274246 BIOSIS
DN BR42:133196
TI ***PURIFICATION*** CHALLENGES FOR RECOMBINANT ***PROTEIN*** C FROM
THE ***MILK*** OF ***TRANSGENIC*** PIGS.
AU VELANDER W H; MORCOL T; DEGENER A; SUBRAMANIAN A
CS DEP. CHEMICAL ENGINEERING, VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIV.,
BLACKSBURG, VA. 24061.
SO 203RD ACS (AMERICAN CHEMICAL SOCIETY) NATIONAL MEETING, SAN FRANCISCO,
CALIFORNIA, USA, APRIL 5-10, 1992. ABSTR PAP AM CHEM SOC. (1992) 203
(1-3), ANYL106.
CODEN: ACSRAL. ISSN: 0065-7727.
DT Conference
FS BR; OLD
LA English

=> d his

(FILE 'HOME' ENTERED AT 14:54:44 ON 09 APR 2002)

FILE 'BIOSIS, MEDLINE, EMBASE' ENTERED AT 14:55:29 ON 09 APR 2002
E MEADE/AU
L1 1 S E3
L2 33 S MEADE H/AU

FILE 'STNGUIDE' ENTERED AT 15:01:59 ON 09 APR 2002

FILE 'BIOSIS, MEDLINE, EMBASE' ENTERED AT 15:18:28 ON 09 APR 2002
L3 26 DUP REM L2 (7 DUPLICATES REMOVED)
L4 4002780 S PROTEIN? OR POLYPEPTIDE?
L5 107841 S L4 (3A) PURIF?
L6 495 S L5 AND TRANSGEN?
L7 20753 S L4 (3A) MILK
L8 160 S L7 (3A) PURIF?
L9 26 S L8 AND TRANSGEN?
L10 15 DUP REM L9 (11 DUPLICATES REMOVED)

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NEWS 5 Feb 19 Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 6 Mar 08 Gene Names now available in BIOSIS
NEWS 7 Mar 22 TOXLIT no longer available
NEWS 8 Mar 22 TRCTHERMO no longer available
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=> FIL BIOSIS MEDLINE EMBASE
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ENTRY SESSION
FULL ESTIMATED COST 0.42 0.42

FILE 'BIOSIS' ENTERED AT 13:26:10 ON 19 APR 2002
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=> s antibody purification
L1 364 ANTIBODY PURIFICATION

=> s l1 < py1999
NUMERIC EXPRESSION NOT VALID 'L1 < PY1999'
NUMERIC EXPRESSION NOT VALID 'L2 < PY1999'
NUMERIC EXPRESSION NOT VALID 'L3 < PY1999'
Numeric search expressions contain an operator (=,>,<,=<,=>), a field qualifier, and the number or a range to be searched. Examples of valid expressions are 'LD>6', '260-280/MW', and '10 < LD < 30'. For a list of field codes in the current file, enter "HELP SFIELDS" at an arrow prompt (=>). For more information on searching in numeric fields, enter "HELP NUMERIC".

=> s l1 and py<1999
1 FILES SEARCHED...
L2 279 L1 AND PY<1999

=> d bib abs 1-10

L2 ANSWER 1 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:67593 BIOSIS
DN PREV200200067593
TI Low pH hydrophobic interaction chromatography for ***antibody*** ***purification*** .
AU Rinderknecht, E. H.; Zapata, G. A.
CS San Carlos, Calif. USA
ASSIGNEE: GENENTECH, INC.
PI US 5641870 June 24, 1997
SO Official Gazette of the United States Patent and Trademark Office Patents, (***June 24, 1997***) Vol. 1199, No. 4, pp. 2676.
ISSN: 0098-1133.
DT Patent
LA English

L2 ANSWER 2 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:29494 BIOSIS
DN PREV200200029494
TI HCG peptides for use in ***antibody*** ***purification*** procedures.
AU Sarin, V. K.; Bodner, J. B.
CS Libertyville, Ill. USA
ASSIGNEE: ABBOTT LABORATORIES
PI US 5451527 Sept. 19, 1995
SO Official Gazette of the United States Patent and Trademark Office Patents, (***Sept. 19, 1995***) Vol. 1178, No. 3, pp. 1664.
ISSN: 0098-1133.
DT Patent
LA English

L2 ANSWER 3 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2002:20271 BIOSIS
DN PREV200200020271
TI ***Antibody*** ***purification*** .
AU Shadle, P. J.; Erickson, J. C.; Scott, R. G.; Smith, T. M.
CS Gulph Mills, Pa. USA
ASSIGNEE: SMITHKLINE BEECHAM CORPORATION
PI US 5429746 July 4, 1995
SO Official Gazette of the United States Patent and Trademark Office Patents, (***July 4, 1995***) Vol. 1176, No. 1, pp. 317.
ISSN: 0098-1133.
DT Patent
LA English

L2 ANSWER 4 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:226404 BIOSIS
DN PREV199900226404
TI ***Antibody*** ***purification*** methods.
AU Vijayalakshmi, M. A. (1)
CS (1) Laboratoire d'Interactions Moleculaires et Technologie des Separations, CNRS-UPRES A 6022, Universite de Technologie de Compiegne, 60205, Compiegne France
SO Applied Biochemistry and Biotechnology, (***Oct., 1998***) Vol. 75, No. 1, pp. 93-102.
ISSN: 0273-2289.
DT Article
LA English
SL English
AB Antibodies (Abs) from the sera of patients with autoimmune diseases are reported to have different catalytic functions. Their recovery by efficient purification methods is, therefore, a crucial step. This article reviews different available methods for their recovery and emphasizes a new approach, namely adsorbents with immobilized histidine, which allows a good purification both in yield and purity of Abs, with the additional advantage of using gentle elution conditions. This, in turn, will ensure the recovery of intact (nondenatured) catalytically functional Abs, directly from the sera.

L2 ANSWER 5 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:226402 BIOSIS
DN PREV199900226402
TI Catalytic DNA- and RNA-hydrolyzing antibodies from milk of healthy human mothers.
AU Buneva, Valentina N.; Kanyshkova, Tat'yana G.; Vlassov, Alexander V.; Semenov, Dmitry V.; Khlimankov, Denis Yu.; Breusova, Lyubov' R.; Nevinsky, Georgy A. (1)
CS (1) Novosibirsk Institute of Bioorganic Chemistry, Siberian Division of Russian Academy of Sciences, Lavrentieva Ave., 8, Novosibirsk, 630090 Russia
SO Applied Biochemistry and Biotechnology, (***Oct., 1998***) Vol. 75, No. 1, pp. 63-76.
ISSN: 0273-2289.
DT Article
LA English
SL English
AB Various catalytically active antibodies (Abs), or abzymes, have been detected recently in the sera of patients with autoimmune pathologies, in whom their presence is probably associated with autoimmunization. Normal humans are generally not considered to have abzymes, since no obvious immunizing factors are present. Here is shown by different methods that IgG from the milk of normal females possesses both DNase and RNase activities. The activities were also present in the IgG F(ab')2 and Fab fragments. Affinity modification of IgG by the chemically reactive derivative of an oligonucleotide led to preferential modification of the L chain of IgG. After separation of the subunits by sodium dodecyl sulfate electrophoresis in a gel containing DNA, an in-gel assay showed DNase activity in the L chain. The L chain separated by affinity chromatography on DNA-cellulose was catalytically active. These findings speak in favor of the generation of catalytic Abs by the immune system of healthy mothers. It is known that the treatment of adults with DNases and RNases offers protection from viral and bacterial diseases. Since breast milk protects the infants from infections until the immune system is developed, this raises the possibility that catalytic Abs like nucleases, may possess a protective role.

L2 ANSWER 6 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:172839 BIOSIS
DN PREV199900172839
TI Preliminary studies on the purification of a monoclonal antibody by affinty precipitation with Eudragit S-100.
AU Taipa, M. Angela (1); Kaul, Rajni; Mattiasson, Bo; Cabral, Joaquim M. S.
CS (1) Cent. Engenharia Biol. Quim., Inst. Superior Tecnico 1000, Lisboa Portugal
SO Journal of Molecular Recognition, (1998) Vol. 11, No. 1-6, pp. 240-242.
ISSN: 0952-3499.
DT Article
LA English
AB A simple procedure for the purification of an IgG-type monoclonal antibody by affinity precipitation using Eudragit S-100 is presented. The ligand, a microbial lipase previously used as antigen, was coupled to the polymer at a concentration of 40 mg lipase/g Eudragit. This macroligand was reversibly precipitated by manipulating the pH at values higher and lower than 4.8. The effects of polymer concentration and dilution of hybridoma culture supernatant on the overall precipitation process were evaluated. The best purification factor was achieved with a polymer concentration of 0.1% (w/v) and a supernatant dilution of 1:3. The preliminary studies reported here enabled the purification of a monoclonal antibody in one step with an activity yield (by ELISA) of 50%-55% and a purification factor of ca 6.

L2 ANSWER 7 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:144959 BIOSIS
DN PREV199900144959
TI IRIS 97: An innovative protein A-peptidomimetic solid phase medium for ***antibody*** ***purification*** .
AU Guerrier, Luc (1); Flayeux, Isabelle; Schwarz, Alex; Fassina, Giorgio; Boschetti, Egisto
CS (1) BioSepra SA, 35 Avenue Jean-Jaures, F-92395 Villeneuve la Garenne France
SO Journal of Molecular Recognition, (1998) Vol. 11, No. 1-6, pp. 107-109.
ISSN: 0952-3499.

DT Article
LA English

L2 ANSWER 8 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:63240 BIOSIS
DN PREV199900063240
TI Development of anti-phenylurea ***antibody*** ***purification*** techniques for improved environmental applications.
AU Rejeb, Samy Ben (1); Durand, Nathalie Fischer; Martel, Annie; Le Poulennec, Bruno; Lawrence, James F.; Hennion, Marie Claire; Le Goffic, Francois
CS (1) Lab. Biotechnol. l'Environ., Ecole Natl. Superieure Chimie Paris, 11, rue Pierre et Marie Curie, 75231 Paris Cedex 05 France
SO Analytica Chimica Acta, (***Dec. 4, 1998***) Vol. 376, No. 1, pp. 41-48.
ISSN: 0003-2670.
DT Article
LA English
AB The nonuniform affinity of polyclonal antibodies is a major problem when these antibodies are used in immunochemical-based environmental applications. A specific affinity chromatography procedure was developed to extract selectively anti-isoproturon antibodies from a crude polyclonal antiserum. These antibodies were fully characterized and helped to improve the performances of immunoaffinity extraction and an indirect enzyme immunoassay.

L2 ANSWER 9 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:63212 BIOSIS
DN PREV199900063212
TI Design of ligands for the purification of anti-MUC1 antibodies by peptide epitope affinity chromatography.
AU Murray, A. (1); Spencer, D. I. R.; Missailidis, S.; Denton, G.; Price, M. R.
CS (1) Cancer Res. Lab., Univ. Nottingham, University Park, Nottingham UK
SO Journal of Peptide Research, (***Nov., 1998***) Vol. 52, No. 5, pp. 375-383.
ISSN: 1397-002X.
DT Article
LA English
AB The fine specificity of epitope recognition of the anti-MUC1 mucin monoclonal antibody, C595 has been studied using solid-phase replacement net (RNET) analysis. Two peptides (RAAP and RPPP) showed increased reactivity with C595 antibody compared with the native epitope (RPAP). These were synthesized as integral motifs within MUC1 immunodominant peptides and analyzed by fluorescence quenching (FQ) and circular dichroism (CD). They were also tested as ligands for the purification of C595 antibody using epitope affinity chromatography. Affinity matrices were compared with respect to capacity, affinity, and quality of the purified product. In FQ tests the native epitope peptide (APDTRPAPG) and the alanine substituted peptide had similar association constants when reacting with C595 antibody, whereas the proline substituted peptide (APDTRPPPG) had a higher association constant. This order of affinity for C595 was confirmed in chromatography experiments in which antibody was eluted from the former two peptide matrices at approximately the same point on the NaSCN elution gradient, whereas antibody was desorbed from APDTRPPPG at a higher NaSCN concentration. Circular dichroism analysis showed that the thermodynamically preferred conformation of these peptides in aqueous solution is the P-II extended helix, the conformation preferred for an extended bound form of the peptide held by interactions with the peptide amides. The stronger binding peptide (APDTRPPPG) has the higher population of the P-II helix in solution. In conclusion, RNET analysis is useful in the rational design of peptide ligands so that the performance of affinity matrices may be regulated.

L2 ANSWER 10 OF 279 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:58930 BIOSIS
DN PREV199900058930
TI Monoclonal antibody to rat CD63 detects different molecular forms in rat tissue.
AU Kennel, S. J. (1); Lankford, P. K.; Foote, L. J.; Davis, I. A.
CS (1) Build. 4500S MS-6101, Life Sci. Div., Oak Ridge Natl. Lab., Oak Ridge, TN 37831-6101 USA
SO Hybridoma, (***Dec., 1998***) Vol. 17, No. 6, pp. 509-515.
ISSN: 0272-457X.
DT Article
LA English
AB From mice immunized with rat endothelial cell membranes, we isolated several hybridomas secreting monoclonal antibodies (MAbs) to a 45-kDa glycoprotein expressed on the surface of cultured cells. One of these antibodies, 523-14A, was purified and used for immunoaffinity chromatography, Western blotting, and immunohistochemistry. The glycoprotein containing the antigen for MAb 523-14A, gp45, was isolated from rat lung endothelial cell membranes using wheat germ agglutinin and antibody affinity chromatography sequentially. Mass spectrometry of tryptic peptides from gel purified bands identified gp45 as rat CD63, a member of the transmembrane-4 superfamily. Western blot analyses of tissues from F344 rats showed that kidney, spleen, uterus, and ovaries expressed CD63 at high levels. Thymus, salivary gland, testicles, intestines, pancreas, and adrenals expressed lower amounts. Tissue cell types expressing CD63 were also examined and the results showed that, in addition to the expected expression on lymphoid cells, CD63 was expressed on many epithelial and muscle cells as well. The mobility of CD63 on SDS-PAGE varied widely, indicative of molecular masses ranging from 45 kDa in some tissues to nearly 60 kDa in others.

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=> s cellulose binding domain
L3 1092 CELLULOSE BINDING DOMAIN

=> s l3 and l1
L4 2 L3 AND L1

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 1 DUP REM L4 (1 DUPLICATE REMOVED)

=> d bib abs

L5 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
AN 2000:313713 BIOSIS
DN PREV200000313713
TI Expression, purification and applications of staphylococcal Protein A fused to ***cellulose*** - ***binding*** ***domain*** .
AU Shpigel, Etai; Goldlust, Arie; Eshel, Adi; Ber, Idit Kaplan; Efroni, Gilat; Singer, Yossi; Levy, Ilan; Dekel, Mara; Shoseyov, Oded (1)
CS (1) Kennedy Leigh Centre for Horticulture Research and Otto Warburg Center for Agricultural Biotechnology, Faculty of Agricultural, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Rehovot, 76100 Israel
SO Biotechnology and Applied Biochemistry, (June, 2000) Vol. 31, No. 3, pp. 197-203. print.
ISSN: 0885-4513.
DT Article
LA English
SL English
AB Because staphylococcal Protein A (ProtA) binds specifically to IgG, it has been used for many immunological manipulations, most notably ***antibody*** ***purification*** and diagnostics. Immobilization is required for most of these applications. Here we describe a genetic-engineering approach to immobilizing ProtA on cellulose, by fusing it to ***cellulose*** - ***binding*** ***domain*** (CBD) derived from the cellulose-binding Protein A of Clostridium cellulovorans. The bifunctional fusion protein was expressed in Escherichia coli, recovered on a cellulose column and purified by elution at alkaline pH. ProtA-CBD was used to purify IgG from rabbit serum and its ability to bind IgG from different sources was determined. The bifunctional chimaeric protein can bind up to 23.4 mg/ml human IgG at a ratio of 1 mol of ProtA-CBD/2 mol of human IgG, and can purify up to 11.6 mg/ml rabbit IgG from a serum. The ability to bind functionally active CBD-affinity reagents to cellulosic microtitre plates was demonstrated. Our results indicate that a combination of CBD-affinity reagents and cellulosic microtitre plates is an attractive diagnostics matrix for the following reasons: (i) cellulose exhibits very low non-specific binding; and (ii) CBD-fusion proteins bind directly to cellulose at high density. A unique signal-amplification method was developed based on the ability of ProtA-CBD to link stained cellulose particles to primary antibody in a Western blot.

=> s antibody (3a) purif?
L6 13521 ANTIBODY (3A) PURIF?

=> s l6 and l3

L7 3 L6 AND L3

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 1 DUP REM L7 (2 DUPLICATES REMOVED)

=> s *chitin binding domain*
L9 266 CHITIN BINDING DOMAIN

=> s l9 and l6
L10 0 L9 AND L6

=> s protein L
L11 2656 PROTEIN L

=> s l11 and l3
L12 1 L11 AND L3

=> d bib abs

L12 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
AN 1992:505710 CAPLUS
DN 117:105710
TI In vitro processing of fusion proteins
IN Heim, Jutta; Seeboth, Peter; Takabayashi, Kenji
PA Ciba-Geigy A.-G., Switz.
SO *Eur. Pat. Appl.*, 35 pp.
CODEN: EPXXDW
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI EP 467839	A1	19920122	EP 1991-810543	19910709
EP 467839	B1	19960821		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 141645	E	19960915	AT 1991-810543	19910709
ES 2091897	T3	19961116	ES 1991-810543	19910709
FI 9103405	A	19920119	FI 1991-3405	19910715
CA 2047119	AA	19920119	CA 1991-2047119	19910716
AU 651830	B2	19940804	AU 1991-80463	19910716
AU 9180463	A1	19920123		
NO 9102803	A	19920120	NO 1991-2803	19910717
HU 58369	A2	19920228	HU 1991-2398	19910717
HU 216104	B	19990428		
ZA 9105579	A	19920325	ZA 1991-5579	19910717
JP 04229188	A2	19920818	JP 1991-176552	19910717
JP 3140488	B2	20010305		

PRAI GB 1990-15825 A 19900718
AB Fusion proteins (P-L)m-T and T-(L-P)n (P = desired biol. active ***protein*** ; ***L*** = linker peptide contg. Lys and/or Arg as *terminal dipeptide*; T = polypeptide tag; *m* = 1-10; *n* = 2-10) are prepd. with transgenic cells, and the desired protein P is prepd. from the fusion protein using sol. yeast gene KEX2 endoprotease yscF and gene KEX1 carboxypeptidase ysc.alpha.. Genes encoding sol. yscF and ysc.alpha. were expressed in Saccharomyces cerevisiae and the enzymes were purified. *Plasmid pDP34GAPDH-eglincex-1, contg. a chimeric gene for eglin C linked* via Lys-Arg-Glu-Ala-Glu-Ala-Trp-Val-Pro to the ***cellulose*** - ***binding*** ***domain*** of the Cellulomonas fimi Exg protein (encoded by the cex gene), was prepd. The fusion protein was produced with S. cerevisiae, purified by cellulose affinity chromatog., and *digested with yscF to remove the Exg protein tag and with ysc.alpha. to* remove the Lys-Arg dipeptide from eglin c.

=> d his

(FILE 'HOME' ENTERED AT 13:24:48 ON 19 APR 2002)

FILE 'BIOSIS, MEDLINE, EMBASE' ENTERED AT 13:26:10 ON 19 APR 2002
L1 364 S ANTIBODY PURIFICATION
L2 279 S L1 AND PY<1999

FILE 'STNGUIDE' ENTERED AT 13:36:11 ON 19 APR 2002

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 13:45:32 ON 19 APR 2002
L3 1092 S CELLULOSE BINDING DOMAIN
L4 2 S L3 AND L1
L5 1 DUP REM L4 (1 DUPLICATE REMOVED)
L6 13521 S ANTIBODY (3A) PURIF?
L7 3 S L6 AND L3
L8 1 DUP REM L7 (2 DUPLICATES REMOVED)
L9 266 S CHITIN BINDING DOMAIN
L10 0 S L9 AND L6
L11 2656 S PROTEIN L
L12 1 S L11 AND L3

=> s l9 and l11
L13 0 L9 AND L11

=> s protein (3a) purif?
L14 96199 PROTEIN (3A) PURIF?

=> s l3 and l14
L15 95 L3 AND L14

=> dup rem l15
PROCESSING COMPLETED FOR L15
L16 56 DUP REM L15 (39 DUPLICATES REMOVED)

=> s l9 and l14
L17 37 L9 AND L14

=> dup rem l17
PROCESSING COMPLETED FOR L17
L18 24 DUP REM L17 (13 DUPLICATES REMOVED)

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 24 ANSWERS - CONTINUE? Y/(N):y

L18 ANSWER 1 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 2002:235420 CAPLUS
TI Design, Production, and Characterization of an Engineered Biotin Ligase (BirA) and Its Application for Affinity Purification of Staphylokinase Produced from Bacillus subtilis via Secretion
AU Wu, Sau-Ching; Yeung, Jonathan C.; Hwang, Peter M.; Wong, Sui-Lam
CS Department of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4, Can.
SO Protein Expression and Purification (2002), 24(3), 357-365
CODEN: PEXPEJ; ISSN: 1046-5928
PB Academic Press
DT Journal
LA English
AB A major attraction in using Bacillus subtilis as an expression host for heterologous protein prodn. is its ability to secrete extracellular proteins into the culture medium. To take full advantage of this system, an efficient method for recovering the target protein is crucial. For secretory proteins which cannot be purified by a simple scheme, in vitro biotinylation using biotin ligase (BirA) offers an effective alternative for their purifn. The availability of large amts. of quality BirA can be crit. for in vitro biotinylation. We report here the engineering and prodn. of an Escherichia coli BirA and its application in the purifn. of staphylokinase, a fibrin-specific plasminogen activator, from the culture supernatant of Bacillus subtilis via in vitro biotinylation. BirA was tagged with both a ***chitin*** - ***binding*** ***domain*** and a hexahistidine tail to facilitate both its purifn. and its removal from the biotinylated sample. We show in this paper how, in a unique way, we solved the problem of protein aggregation in the E. coli BirA prodn. system to achieve a yield of sol. functional BirA hitherto unreported in the literature. Application of this novel BirA to ***protein*** ***purifn*** . via in vitro biotinylation in general will also be discussed. Biotinylated staphylokinase produced in the study not only can act as an intermediate for easy purifn., it can also serve as an important element in the creation of a blood clot targeting and dissolving agent. (c) 2002 Academic Press.

L18 ANSWER 2 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2002013048 EMBASE
TI Identification and characterization of the gene cluster involved in chitin degradation in a marine bacterium, Alteromonas sp. strain O-7.
AU Tsujibo H.; Orikoshi H.; Baba N.; Miyahara M.; Miyamoto K.; Yasuda M.; Inamori Y.
CS H. Tsujibo, Osaka Univ. of Pharmaceutical Sci., 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan. tsujibo@gly.oups.ac.jp
SO Applied and Environmental Microbiology, (2002) 68/1 (263-270).
Refs: 39
ISSN: 0099-2240 CODEN: AEMIDF
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Alteromonas sp. strain O-7 secretes chitinase A (ChiA), chitinase B (ChiB), and chitinase C (ChiC) in the presence of chitin. A gene cluster involved in the chitinolytic system of the strain was cloned and sequenced upstream of and including the chiA gene. The gene cluster consisted of three different open reading frames organized in the order chiD, cbp1, and chiA. The chiD, cbp1, and chiA genes were closely linked and transcribed in the same direction. Sequence analysis indicated that Cbp1 (475 amino acids) was a chitin-binding protein composed of two discrete functional regions. ChiD (1,037 amino acids) showed sequence similarity to bacterial chitinases classified into family 18 of glycosyl hydrolases. The cbp1 and chid genes were expressed in Escherichia coli, and the recombinant proteins were purified to homogeneity. The highest binding activities of Cbp1 and ChiD were observed when .alpha.-chitin was used as a substrate. Cbp1 and ChiD possessed a ***chitin*** - ***binding*** ***domain*** (ChtBD) belonging to ChtBD type 3. ChiD rapidly hydrolyzed chitin oligosaccharides in sizes from trimers to hexamers, but not chitin. However, after prolonged incubation with large amounts of ChiD, the enzyme produced a small amount of (GlcNAc)(2) from chitin. The optimum temperature and pH of ChiD were 50.degree.C and 7.0, respectively.

L18 ANSWER 3 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2001077914 EMBASE
TI Cloning, sequences, and characterization of two chitinase genes from the Antarctic Arthrobacter sp. Strain TAD20: Isolation and partial

characterization of the enzymes.
AU Lonhienne T.; Mavromatis K.; Vorgias C.E.; Buchon L.; Gerday C.; Bouriotis V.
CS V. Bouriotis, Department of Biology, Div. of Appl. Biology/Biotechnology, University of Crete, P.O. Box 1470, Heraklion 71110, Crete, Greece. bouriotis@imbb.forth.gr
SO Journal of Bacteriology, (2001) 183/5 (1773-1779).
Refs: 37
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
022 Human Genetics
LA English
SL English
AB Arthrobacter sp. strain TAD20, a chitinolytic gram-positive organism, was isolated from the sea bottom along the Antarctic ice shell. Arthrobacter sp. strain TAD20 secretes two major chitinases, ChiA and ChiB (ArChiA and ArChiB), in response to chitin induction. A single chromosomal DNA fragment containing the genes coding for both chitinases was cloned in Escherichia coli. DNA sequencing analysis of this fragment revealed two contiguous open reading frames coding for the precursors of ArChiA (881 amino acids [aa]) and ArChiB (578 aa). ArChiA and ArChiB are modular enzymes consisting of a glycosyl-hydrolase family 18 catalytic domain as well as two and one chitin-binding domains, respectively. The catalytic domain of ArChiA exhibits 55% identity with a chitodextrinase from Vibrio furnissii. The ArChiB catalytic domain exhibits 33% identity with chitinase A of Bacillus circulans. The ArChiA chitin-binding domains are homologous to the ***chitin*** - ***binding*** ***domain*** of ArChiB. ArChiA and ArChiB were purified to homogeneity from the native Arthrobacter strain and partially characterized. Thermal unfolding of ArChiA, ArChiB, and chitinase A of Serratia marcescens was studied using differential scanning calorimetry. ArChiA and ArChiB, compared to their mesophilic counterpart, exhibited increased heat lability, similar to other cold-adapted enzymes.

L18 ANSWER 4 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
1
AN 2001:503759 BIOSIS
DN PREV200100503759
TI A conserved domain in arthropod cuticular proteins binds chitin.
AU Rebers, John E. (1); Willis, Judith H.
CS (1) Department of Biology, Northern Michigan University, Marquette, MI, 49855: jrebers@nmu.edu USA
SO Insect Biochemistry and Molecular Biology, (October, 2001) Vol. 31, No. 11, pp. 1083-1093. print.
ISSN: 0965-1748.
DT Article
LA English
SL English
AB Many insect cuticular proteins include a 35-36 amino acid motif known as the R&R consensus. The extensive conservation of this region led to the suggestion that it functions to bind chitin. Provocatively, it has no sequence similarity to the well-known cysteine-containing ***chitin*** - ***binding*** ***domain*** found in chitinases and some peritrophic membrane proteins. Using fusion proteins expressed in E. coli, we show that an extended form of the R&R consensus from proteins of hard cuticles is necessary and sufficient for chitin binding. Recombinant AGCP2b, a putative cuticular protein from the mosquito Anopheles gambiae, was expressed in E. coli and the ***purified*** ***protein*** shown to bind to chitin beads. A stretch of 65 amino acids from AGCP2b, including the R&R consensus, conferred chitin binding to glutathione-S-transferase (GST). Directed mutagenesis of some conserved amino acids within this extended R&R consensus from hard cuticle eliminated chitin binding. Thus arthropods have two distinct classes of chitin binding proteins, those with the ***chitin*** - ***binding*** ***domain*** found in lectins, chitinases and peritrophic membranes (cysCBD) and those with the cuticular protein ***chitin*** - ***binding*** ***domain*** (non-cysCBD).

L18 ANSWER 5 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 2
AN 2001228468 EMBASE
TI Reconstitution and purification of cytolethal distending toxin of Actinobacillus actinomycetemcomitans.
AU Saiki K.; Konishi K.; Gomi T.; Nishihara T.; Yoshikawa M.
CS Dr. K. Saiki, Department of Microbiology, Nippon Dental Univ. of Sch. Dent., 1-9-20 Fujimi, Chiyoda-ku, Tokyo 102-8159, Japan. keisaiki@tokyo.ndu.ac.jp
SO Microbiology and Immunology, (2001) 45/6 (497-506).
Refs: 27
ISSN: 0385-5600 CODEN: MIIMDV
CY Japan
DT Journal; General Review
FS 004 Microbiology
LA English
SL English
AB Cytolethal distending toxin (CDT) has been found in various pathogenic bacterial species and causes a cell distending and a G(2) arrest against eukaryotic cells. All the cdtABC genes, which encode CDT, are known to be required for the CDT activities although the CDT holotoxin structure has not been elucidated. We cloned the cdtABC genes of Actinobacillus actinomycetemcomitans and constructed an Escherichia coli expression system for them. We found that crude extracts from six deletion mutants (.DELTA.cdtA, .DELTA.cdtB, .DELTA.cdtC, .DELTA.cdtBC, .DELTA.cdtAC, and .DELTA.cdtAB) of recombinant E. coli, which showed very weak or no detectable CDT activities, restored the CDT activities when pre-mixing and pre-incubation of them were performed in combinations to contain all the CdtA, CdtB, and CdtC proteins. These results indicate that all the Cdt proteins are required for the CDT activities. We also found that the chimera CdtB protein, CdtB-intein-CBD (***chitin*** ***binding*** ***domain***) like CdtB protein itself assembled with CdtA and CdtC. The reconstituted CDT containing the chimera CdtB protein was specifically extracted by chitin beads and the only CDT portion was isolated from the chitin beads by a cleavage reaction of the intein. The purified reconstituted-CDT was found to consist of CdtA, CdtB, and CdtC proteins, and showed appreciable CDT activities, indicating that the CDT holotoxin structure is the CdtABC complex. To our knowledge, this is the first report succeeded in complete purification of an active CDT and may offer useful tools for elucidation of the toxic mechanism of CDT.

L18 ANSWER 6 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
3
AN 2001:420802 BIOSIS
DN PREV200100420802
TI Cloning, expression, and purification of the Staphylococcus simulans lysostaphin using the intein- ***chitin*** - ***binding*** ***domain*** (CBD) system.
AU Szweda, Piotr; Pladzyk, Radoslaw; Kotlowski, Roman; Kur, Jozef (1)
CS (1) Department of Microbiology, Technical University of Gdansk, Ul. Narutowicza 11/12, 80-952, Gdansk: kur@altis.chem.pg.gda.pl Poland
SO Protein Expression and Purification, (August, 2001) Vol. 22, No. 3, pp. 467-471. print.
ISSN: 1046-5928.
DT Article
LA English
SL English
AB The Staphylococcus simulans gene encoding lysostaphin has been PCR amplified from pRG5 recombinant plasmid (ATCC 67076) and cloned into Escherichia coli expression pTYB12 vector (IMPACT-CN System, New England BioLabs) which allows the overexpression of a target protein as a fusion to a self-cleavable affinity tag. The self-cleavage activity of the intein allows the release of the lysostaphin enzyme from the chitin-bound intein tag, resulting in a single-column ***purification*** of the target ***protein*** . This abundant over-production allows purifying milligram amounts of the enzyme.

L18 ANSWER 7 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 2001:164270 CAPLUS
DN 134:337725
TI Purification of Eukaryotic MutL Homologs from Saccharomyces cerevisiae Using Self-Cleaving Affinity Technology
AU Hall, Mark C.; Kunkel, Thomas A.
CS Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC, 27709, USA
SO Protein Expression and Purification (2001), 21(2), 333-342
CODEN: PEXPEJ; ISSN: 1046-5928
PB Academic Press
DT Journal
LA English
AB Self-cleaving affinity technol. is an effective tool for rapid purifn. of native sequence recombinant proteins overproduced in Escherichia coli. In this report, we describe the adaptation of this technol. to purify DNA mismatch repair proteins overproduced in the eukaryote Saccharomyces cerevisiae. Mlh1 and Pms1 are homologs of the E. coli MutL protein that participate in a variety of DNA transactions in cells, including correction of DNA replication errors, recombination, excision repair, and checkpoint control. Difficulties in prepg. substantial quantities of highly purified MutL homologs have impeded descriptions of their biophys. and biochem. properties and mechanisms of action. To overcome this limitation, here we use self-cleaving affinity technol. to purify to apparent homogeneity the yeast Mlh1-Pms1 heterodimer and the individual yeast and human Mlh1 subunit. The availability of these proteins should accelerate an understanding of their multiple functions in mismatch repair and other DNA transactions. The general approach is a valid alternative for simple, rapid purifn. of recombinant proteins in yeast when expression in bacteria is unsuitable. (c) 2001 Academic Press.
RE.CNT 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 8 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 2001:613447 CAPLUS
DN 136:211463
TI Cleavage and purification of intein fusion proteins using the Streptococcus gordonii SPEX system
AU Myscofski, Dawn M.; Dutton, Emma K.; Cantor, Eric; Zhang, Aihua; Hruby, Dennis E.
CS Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR, 97331-3804, USA
SO Preparative Biochemistry & Biotechnology (2001), 31(3), 275-290
CODEN: PBBIF4; ISSN: 1082-6068
PB Marcel Dekker, Inc.
DT Journal

LA English
AB A Gram-pos. bacterial expression vector using Streptococcus gordonii has been developed for expression and secretion, or surface anchoring of heterologous proteins. This system, termed Surface Protein Expression system or SPEX, has been used to express a variety of surface anchored and secreted proteins. In this study, the Mycobacterium xenopi (Mxe) GyrA intein and ***chitin*** ***binding*** ***domain*** from Bacillus circulans chitinase A1 were used in conjunction with SPEX to express a fusion protein to facilitate secretion, cleavage, and purifn. Streptococcus gordonii was transformed to express a secreted fusion protein consisting of a target protein with a C-terminal intein and ***chitin*** - ***binding*** ***domain*** . Two target proteins, the C-repeat region of the Streptococcus pyogenes M6 protein (M6) and the nuclease A (NucA) enzyme of Staphylococcus aureus, were expressed and tested for intein cleavage. The secreted fusion proteins were purified from culture medium by binding to chitin beads and subjected to reaction conditions to induce intein self-cleavage to release the target protein. The M6 and NucA fusion proteins were shown to bind chitin beads and elute under cleavage reaction conditions. In addn., NucA demonstrated enzyme activity both before and after intein cleavage.
RE.CNT 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 9 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
AN 2001:525128 BIOSIS
DN PREV200100525128
TI A method for expression and purification of soluble, active Hsp47, a collagen-specific molecular chaperone.
AU Thomson, Christy A.; Ananthanarayanan, Vettai S. (1)
CS (1) Department of Biochemistry, McMaster University, Hamilton, ON, L8N 3Z5: ananth@mcmaster.ca USA
SO Protein Expression and Purification, (October, 2001) Vol. 23, No. 1, pp. 8-13. print.
ISSN: 1046-5928.
DT Article
LA English
SL English
AB Hsp47 is regarded as a collagen-specific chaperone with several suggested roles in collagen biosynthesis under normal and disease conditions. We describe here a procedure for the expression and purification of Hsp47 in Escherichia coli using the IMPACT expression system (New England Biolabs) where the guest gene is fused to the adduct, intein, with a ***chitin*** - ***binding*** ***domain*** . Use of this system resulted in relatively high levels of soluble Hsp47 compared to other available protocols, especially when the bacterial cells were induced at 14degreeC instead of 37degreeC. The cell lysate was passed through a chitin-Sepharose affinity column and Hsp47 was cleaved from intein using beta-mercaptoethanol. Minor degradation products were subsequently removed using a hydroxylapatite column to yield milligram amounts of pure and active protein suitable for structural studies. Gel electrophoretic analysis of the ***purified*** ***protein*** indicated the presence of a small proportion of trimeric species when non-reducing conditions were used. The ability to form a trimer may be important for its role as a chaperone. The IMPACT system allows for radiolabelling of purified Hsp47 with 35S for use in binding experiments. Illustrative data on collagen binding by 35S-Hsp47 are shown.

L18 ANSWER 10 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 2000:820529 CAPLUS
DN 134:112519
TI Fusions to self-splicing inteins for ***protein*** ***purification***
AU Xu, Ming-Qun; Paulus, Henry; Chong, Shaorong
CS New England Biolabs, Inc., Beverly, MA, 01915, USA
SO Methods in Enzymology (2000), 326(Applications of Chimeric Genes and Hybrid Proteins, Pt. A), 376-418
CODEN: MENZAU; ISSN: 0076-6879
PB Academic Press
DT Journal
LA English
AB Protein splicing involves the self-catalyzed excision of an intervening polypeptide segment, the intein, from a precursor protein, with the concomitant ligation of the flanking polypeptide sequence, the exteins, to yield a functional protein. The catalysis of protein splicing is entirely mediated by the intein and involves three distinct reaction steps. Elucidation of the sequence of steps that underlie protein splicing and studies on the effect of amino acid substitutions in the intein and adjacent residues on these steps led to the realization that catalysis of each of the steps in the protein splicing pathway is relatively independent and opened the way for modulating the protein splicing process as a protein engineering tool. It is described how inteins can be used to effect the self-catalyzed cleavage of fusion proteins at highly specific sites. Also, the mechanism of protein splicing in the context of the amino acid residues surrounding the splice junctions is briefly reviewed. (c) 2000 Academic Press.
RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 11 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
AN 2001:49080 BIOSIS
DN PREV200100049080
TI Analysis of three overexpression systems for VanX, the Zinc(II) dipeptidase required for high-level vancomycin resistance in bacteria.
AU Brandt, Jeffrey J.; Chatwood, Lisa L.; Crowder, Michael W. (1)
CS (1) Department of Chemistry and Biochemistry, Miami University, 112 Hughes Hall, Oxford, OH, 45056: crowdermw@muohio.edu USA
SO Protein Expression and Purification, (November, 2000) Vol. 20, No. 2, pp. 300-307. print.
ISSN: 1046-5928.
DT Article
LA English
SL English
AB The gene from Enterococcus faecilis encoding the dipeptidase VanX was subcloned into overexpression vectors pET-5b, pET-27b, and IMPACT-T7, and VanX was overexpressed in BL21(DE3) pLysS. Escherichia coli. The pET-5b-vanx overexpression plasmid produces VanX at apprx12 mg/L under optimum conditions. VanX produced from this overexpression system exists primarily as a dimer in solution, binds ca. 1 Zn(II) ion per monomer, and exhibits Km and kcat values of 500 +- 40 muM and 0.074 +- 0.001 s-1, respectively, when L-alanine-p-nitroanilide is used as substrate. The IMPACT-T7-vanx overexpression plasmid produces a VanX-fusion protein with a ***chitin*** - ***binding*** ***domain*** that allows for purification of the fusion construct with a chitin column. Cleavage of the fusion protein is completed by an on-column chemical cleavage, resulting in apprx10 mg/L of purified VanX. VanX produced from this system is identical to that produced from the pET-5b system, except the CD spectrum of the IMPACT-T7-produced VanX suggests a small change in secondary structure. This change in secondary structure does not affect any of the kinetic or metal-binding properties of the enzyme. The pET-27b-vanx overexpression plasmid produces and secretes VanX into the growth medium; this system allows for 20 mg of VanX to be isolated per liter of growth medium. The pET-27b-produced VanX is identical to that produced from pET-5b.

L18 ANSWER 12 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 6
AN 2001009005 EMBASE
TI Single-column purification and bio-characterization of recombinant human parathyroid hormone-related protein (1-139).
AU Wu C.; Seitz P.K.; Falzon M.
CS M. Falzon, Department Pharmacology/Toxicology, University of Texas Medical Branch, 10th and Market Streets, Galveston, TX 77555-1031, United States. mfalzon@utmb.edu
SO Molecular and Cellular Endocrinology, (22 Dec 2000) 170/1-2 (163-174).
Refs: 40
ISSN: 0303-7207 CODEN: MCEND6
PUI S 0303-7207(00)00323-3
CY Ireland
DT Journal; Article
FS 016 Cancer
029 Clinical Biochemistry
003 Endocrinology
037 Drug Literature Index
LA English
SL English
AB Recombinant human parathyroid hormone-related protein (hPTHrP) (1-139) was expressed using the IMPACT T7 (intein-mediated purification with an affinity chitin-binding tag) system, allowing purification of free recombinant peptide in a single chromatographic step. This system utilizes an intein, which is a protein splicing element from the Saccharomyces cerevisiae VMA1 gene. The intein has been modified so that it undergoes a self-cleavage reaction at its N-terminus at low temperatures in the presence of 1,4-dithiothreitol (DTT). The cDNA encoding hPTHrP (1-139) was cloned into the pTYB1 vector to create an in-frame fusion at the N-terminus of the intein gene. The cDNA for the ***chitin*** - ***binding*** ***domain*** from Bacillus circulans is present at the C-terminus of intein for affinity purification of the three-part fusion protein on a chitin column. The recombinant plasmid was transfected into E. coli ER2566 cells and synthesis of the PTHrP fusion protein was induced with isopropyl-.beta.-D-thiogalactopyranoside (IPTG). This system produced pure hPTHrP (1-139) and an N-terminally truncated analogue, hPTHrP (27-139), as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis, Western blot analysis, N-terminal sequence analysis and mass spectroscopy. hPTHrP (1-139) stimulated cAMP accumulation in ROS 17/2.8 osteoblastic bone cells, whereas hPTHrP (27-139) failed to elicit a response. hPTHrP (1-139) also inhibited the growth of the breast cancer cell line MDA-MB-231; the magnitude of the response was comparable with that of synthetic hPTHrP (1-34) and (1-86). Neutralization of endogenous PTHrP and added hPTHrP (1-139) and N-terminal species with an anti-PTHrP antiserum completely abolished the growth inhibitory effects. These results indicate that the added peptides modulate cell growth by acting at the cell surface. Availability of recombinant hPTHrP (1-139) will allow further study of its biological function, as well as its structure.

L18 ANSWER 13 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 2000:167989 CAPLUS
DN 133:295398

TI Construction of the over expression system of fibronectin type III-like domain and its interactivity with chitin
AU Hashimoto, Masayuki; Shida, Keiko; Watanabe, Takeshi
CS Department of Applied Biological Chemistry, Faculty of Agriculture, Niigata University, Ikarashi-2, Niigata, 950-2181, Japan
SO Kichin, Kitosan Kenkyu (2000), 6(1), 1-8
CODEN: KKKEFB; ISSN: 1340-9778
PB Nippon Kichin, Kitosan Gakkai
DT Journal
LA Japanese
AB Chitinase AI from Bacillus circulant WL-12 comprises four discrete domains, namely, an N-terminal catalytic domain, two fibronectin type III-like domains (FnIII domains) and a C-terminal ***chitin*** ***binding*** ***domain*** . The FnIII domains are the domains homologous to the type Im homol. units of fibronectin, a higher eucaryotic protein with multifunctional activity. To study structure and function of FnIII domains of chitinase AI, Escherichia coli expression system of isolated FnIII domain was constructed by using pET system. The construction was designed to add histidine tag consisted of ten histidine residues to the N-terminus of the FnEII domain. FnIII domain produced in E. coli cells was purified from sol. fraction of disrupted cells by ammonium sulfate pptn., HPLC with DEAE column and affinity chromatog. with His-Bind column. The ***purified*** prepn. exhibited single ***protein*** band in SDS-PAGE anal. From one litter culture of E. coli cells, approx. 10 mg of purified FnIII domain was finally obtained. The purified FnIII domain did not show any significant binding activity to insol. chitin.

L18 ANSWER 14 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 7
AN 1999:455640 BIOSIS
DN PREV199900455640
TI Characterization of a Pseudomonas aeruginosa fatty acid biosynthetic gene cluster: ***Purification*** of acyl carrier ***protein*** (ACP) and malonyl-coenzyme A:ACP transacylase (FabD.
AU Kutchma, Alecksandr J.; Hoang, Tung T.; Schweizer, Herbert P. (1)
CS (1) Department of Microbiology, Colorado State University, Fort Collins, CO, 80523 USA
SO Journal of Bacteriology, (Sept., 1999) Vol. 181, No. 17, pp. 5498-5504. ISSN: 0021-9193.
DT Article
LA English
SL English
AB A DNA fragment containing the Pseudomonas aeruginosa fabD (encoding malonyl-coenzyme A (CoA):acyl carrier protein (ACP) transacylase), fabG (encoding beta-ketoacyl-ACP reductase), acpP (encoding ACP), and fabF (encoding beta-ketoacyl-ACP synthase II) genes was cloned and sequenced. This fab gene cluster is delimited by the plsX (encoding a poorly understood enzyme of phospholipid metabolism) and pabC (encoding 4-amino-4-deoxychorismate lyase) genes; the fabF and pabC genes seem to be translationally coupled. The fabH gene (encoding beta-ketoacyl-ACP synthase III), which in most gram-negative bacteria is located between plsX and fabD, is absent from this gene cluster. A chromosomal temperature-sensitive fabD mutant was obtained by site-directed mutagenesis that resulted in a W258Q change. A chromosomal fabF insertion mutant was generated, and the resulting mutant strain contained substantially reduced levels of cis-vaccenic acid. Multiple attempts aimed at disruption of the chromosomal fabG gene were unsuccessful. We purified FabD as a hexahistidine fusion protein (H6-FabD) and ACP in its native form via an ACP-intein- ***chitin*** ***binding*** ***domain*** fusion protein, using a novel expression and purification scheme that should be applicable to ACP from other bacteria. Matrix-assisted laser desorption-ionization spectroscopy, native polyacrylamide electrophoresis, and amino-terminal sequencing revealed that (i) most of the purified ACP was properly modified with its 4'-phosphopantetheine functional group, (ii) it was not acylated, and (iii) the amino-terminal methionine was removed. In an in vitro system, purified ACP functioned as acyl acceptor and H6-FabD exhibited malonyl-CoA:ACP transacylase activity.

L18 ANSWER 15 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1999339495 EMBASE
TI Construction and use of low-copy number T7 expression vectors for purification of problem proteins: Purification of Mycobacterium tuberculosis RmlD and Pseudomonas aeruginosa LasI and RhlI proteins, and functional analysis of purified RhlI.
AU Hoang T.T.; Ma Y.; Stern R.J.; McNeil M.R.; Schweizer H.P.
CS H.P. Schweizer, Department of Microbiology, Colorado State University, Fort Collins, CO 80523-1677, United States. hschweiz@cvmbs.colostate.edu
SO Gene, (1999) 237/2 (361-371).
Refs: 38
ISSN: 0378-1119 CODEN: GENED6
PUI S 0378-1119(99)00331-5
CY Netherlands
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Purification of proteins from Escherichia coli under native conditions is often hampered by inclusion-body formation after overexpression from T7 promoter-based expression vectors. This is probably due to the relatively high copy number of the ColE1-based expression vectors. To circumvent these problems, the low-copy-number pViet and pNam expression vectors were constructed. These vectors contain the pSC101 origin of replication and allow the expression of oligohistidine and intein ***chitin*** - ***binding*** ***domain*** fusion proteins, respectively. Since pViet and pNam do not replicate in E. coli B strains, an E. coli K-12 host strain [SA1503(DE3)] was constructed. This strain is defective in the Lon and OmpT proteases and allows IPTG-inducible expression of recombinant proteins from the T7 promoter. The new vectors were successfully tested by purification of three very insoluble proteins (RmlD, LasI and RhlI) under non-denaturing conditions, and all three proteins retained enzymatic activity. The purified hexahistidine (His6)-tagged Pseudomonas aeruginosa RhlI protein was subjected to more detailed analyses, which indicated that (1) only butyryl-acyl carrier protein (ACP) and S-adenosylmethionine (SAM) were required for synthesis of N-butyryl-L-homoserine lactone; (2) when present at physiological concentrations, butyryl-coenzyme A and NADPH were not substrates for RhlI; (3) RhlI was able to synthesize N-hexanoyl-L-homoserine lactone from hexanoyl-ACP and SAM; (4) RhlI was able to direct synthesis of N-butyryl-L-homoserine lactone from crotonyl-ACP in a reaction coupled to purified P. aeruginosa FabI (enoyl-ACP reductase).

L18 ANSWER 16 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 1999:202250 CAPLUS
DN 131:1957
TI A new protein splicing system and its splicing conditions
AU Shi, Yawei; Fan, Junhu; Li, Zhuoyu; Yuan, Jingming; Zu, Mingqun; Zhong, Shaorong
CS Biotechnology Center, Shanxi Univ., Taiyuan, 030006, Peop. Rep. China
SO Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1999), 15(1), 88-91
CODEN: ZSHXF2; ISSN: 1007-7626
PB Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
DT Journal
LA Chinese
AB For the mechanism of protein splicing, the splicing conditions for a precursor protein expressed by E. coli 2426/pMYB129 were studied. The expression product, maltose binding protein- ***chitin*** ***binding*** ***domain*** (MYB) was purified by one step on amylose column. Though the precursor protein could self-splice it was quite stable at 4.degree.C. However, the rate of cleavage for precursor could be swiftly increased in the presence of reducing reagent such as dTT, CySH, .beta.-ME, etc. and the rate of cleavage was DTT > CySH > .beta.-ME. According to the quantity of maltose binding protein spliced at the different time on SDS-PAGE, the rate const. of cleavage for DTT: k = 6 .times. 10-3 min-1 (assuming first order kinetics). The rate of protein splicing was affected by temp. and pH. It uses the principle of ***protein*** splicing to ***purify*** recombinant proteins, it would be of some advantages as follows: (a) chem. cleavage could replace enzymic digestion due to having a splicing site at the N-terminal of intein. (b) It could make the N-terminal of the target protein free, because the cleavage site of the precursor was designed at the C-terminal of the target protein. (c) Cleavage could be performed on an affinity chitin column by one step. The system including intein-CBD could become a new methods in the purifn. of recombinant proteins.

L18 ANSWER 17 OF 24 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1999231830 EMBASE
TI Characterization of a self-splicing mini-intein and its conversion into autocatalytic N- and C-terminal cleavage elements: Facile production of protein building blocks for protein ligation.
AU Mathys S.; Evans T.C. Jr.; Chute I.C.; Wu H.; Chong S.; Benner J.; Liu X.-Q.; Xu M.-Q.
CS M.-Q. Xu, New England Biolabs, Inc., 32 Tozer Road, Beverly, MA 01915, United States. xum@neb.com
SO Gene, (29 Apr 1999) 231/1-2 (1-13).
Refs: 35
ISSN: 0378-1119 CODEN: GENED6
PUI S 0378-1119(99)00103-1
CY Netherlands
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The determinants governing the self-catalyzed splicing and cleavage events by a mini-intein of 154 amino acids, derived from the dnaB gene of Synechocystis sp. were investigated. The residues at the splice junctions have a profound effect on splicing and peptide bond cleavage at either the N- or C-terminus of the intein. Mutation of the native Gly residue preceding the intein blocked splicing and cleavage at the N-terminal splice junction, while substitution of the intein C-terminal Asn154 resulted in the modulation of N-terminal cleavage activity. Controlled cleavage at the C- terminal splice junction involving cyclization of Asn154 was achieved by substitution of the intein N-terminal cysteine residue with alanine and mutation of the native C-extein residues. The C-terminal cleavage reaction was found to be pH-dependent, with an optimum between pH 6.0 and 7.5. These findings allowed the development of single junction cleavage vectors for the facile production of proteins as well as protein building blocks with complementary reactive groups. A protein sequence was fused to either the N- terminus or C-terminus of the intein, which was fused to a ***chitin*** ***binding*** ***domain*** . The N-terminal cleavage reaction was induced by 2- mercaptoethanesulfonic acid and released the 43 kDa maltose binding protein with an active C-terminal thioester. The 58 kDa T4 DNA ligase possessing an N-terminal cysteine was generated by a C-terminal cleavage reaction induced by pH and temperature shifts. The intein-generated proteins were joined together

through a native peptide bond. This intein-mediated protein ligation approach opens up novel routes in protein engineering.

L18 ANSWER 18 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
8
AN 1997:340074 BIOSIS
DN PREV199799639277
TI Single-column purification of free recombinant proteins using a self-cleavable affinity tag derived from a protein splicing element.
AU Chong, Shaorong; Mersha, Fana B.; Comb, Donald G.; Scott, Melissa E.; Landry, David; Vence, Luis M.; Perler, Francine B.; Benner, Jack; Kucera, Rebecca B.; Hirvonen, Christine A.; Pelletier, John J.; Paulus, Henry; Xu, Ming-Qun (1)
CS (1) New England Biolabs, 32 Tozer Rd., Beverly, MA 01915 USA
SO Gene (Amsterdam), (1997) Vol. 192, No. 2, pp. 271-281.
ISSN: 0378-1119.
DT Article
LA English
AB A novel ***protein*** ***purification*** system has been developed which enables purification of free recombinant proteins in a single chromatographic step. The system utilizes a modified protein splicing element (intein) from Saccharomyces cerevisiae (Sce VMA intein) in conjunction with a ***chitin*** - ***binding*** ***domain*** (CBD) from Bacillus circulans as an affinity tag. The concept is based on the observation that the modified Sce VMA intein can be induced to undergo a self-cleavage reaction at its N-terminal peptide linkage by 1,4-dithiothreitol (DTT), beta-mercaptoethanol (beta-ME) or cysteine at low temperatures and over a broad pH range. A target protein is cloned in-frame with the N-terminus of the intein-CBD fusion, and the stable fusion ***protein*** is ***purified*** by adsorption onto a chitin column. The immobilized fusion protein is then induced to undergo self-cleavage under mild conditions, resulting in the release of the target protein while the intein-CBD fusion remains bound to the column. No exogenous proteolytic cleavage is needed. Furthermore, using this procedure, the ***purified*** free target ***protein*** can be specifically labeled at its C-terminus.

L18 ANSWER 19 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
9
AN 1996:468512 BIOSIS
DN PREV199699190868
TI Novel osmotically induced antifungal chitinases and bacterial expression of an active recombinant isoform.
AU Yun, Dae-Jin; D'Urzo, Matilde Paino; Abad, Laura; Takeda, Satomi; Salzman, Ron; Chen, Zutang; Lee, Hyeseung; Hasegawa, Paul M.; Bressan, Ray A. (1)
CS (1) Cent. Plant Environmental Stress Physiol., 1165 Horticulture Build., Purdue Univ., West Lafayette, Indiana 47907-1165 USA
SO Plant Physiology (Rockville), (1996) Vol. 111, No. 4, pp. 1219-1225.
ISSN: 0032-0889.
DT Article
LA English
AB NaCl (428 mM)-adapted tobacco (Nicotiana tabacum L. var Wisconsin 38) cells accumulate and secrete several antifungal chitinases. The predominant protein secreted to the culture medium was a 29-kD peptide that, based on internal amino acid sequence, was determined to be a class II acidic chitinase with similarity to PR-Q. The four predominant chitinases (T1, T2, T3, and T4) that accumulated intracellularly in 428 mm NaCl-adapted cells were purified. Based on N-terminal sequence analyses, two of these were identified as class I chitinase isoforms, one similar to the N. tomentosiformis (H. Shinshi, J.M. Neuhaus, J. Ryals, F. Meins (1990) Plant Mol Biol 14: 357-368) protein (T1) and the other homologous to the N. sylvestris (Y. Fukuda, M. Ohme, H. Shinshi (1 991) Plant Mol Biol 16: 1-10) protein (T2). The other two proteins (T3 and T4) were determined to be novel chitinases that have sequence similarity with class I chitinases, but each lacks a ***chitin*** - ***binding*** ***domain*** . All four chitinases inhibited Fusarium oxysporum f. sp. lycopersici and Trichoderma longibrachiatum hyphal growth in vitro, although the isoforms containing a ***chitin*** - ***binding*** ***domain*** were somewhat more active. Conditions were established for the successful expression of soluble and active bacterial recombinant T2. Expression of soluble recombinant T2 was achieved when isopropyl beta-D-thiogalactopyranoside induction occurred at 18 degree C but not at 25 or 37 degree C. The ***purified*** recombinant ***protein*** exhibited antifungal activity comparable to a class I chitinase purified from NaCl-adapted tobacco cells.

L18 ANSWER 20 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
10
AN 1996:161975 BIOSIS
DN PREV199698734110
TI Purification of a Zn-binding phloem protein with sequence identity to chitin-binding proteins.
AU Taylor, Kathryn C. (1); Albrigo, L. Gene; Chase, Christine D.
CS (1) Dep. Plant Sci., Univ. Arizona, Tucson, AZ 85721 USA
SO Plant Physiology (Rockville), (1996) Vol. 110, No. 2, pp. 657-664.
ISSN: 0032-0889.
DT Article
LA English
AB In citrus blight, a decline disorder of unknown etiology, the tree canopy exhibits symptoms of Zn deficiency while Zn accumulates in the trunk phloem. We have ***purified*** a Zn-binding ***protein*** (ZBP) from phloem tissue of healthy and blight-affected citrus (Citrus sinensis (L.) Osbeck on Citrus jambhiri (L.)). The molecular weight of the ZBP was estimated to be 5000 by size-exclusion chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Ion-exchange chromatography at pH 8.0 demonstrated the 5-kD ZBP to be anionic. A partial N-terminal amino acid sequence revealed a cysteine-, glycine-rich domain with 45 to 80% identity with the ***chitin*** - ***binding*** ***domain*** of hevein, wheat germ agglutinin, and several class I chitinases. That the abundance of this protein increased 2.5-fold in association with Zn accumulation in the phloem is characteristic of citrus blight. Tissue mass changes of the phloem suggests that altered tissue structure accompanies blight. Phloem accumulation of the 5-kD ZBP may be in response to wounding or other stress of blight-affected citrus.

L18 ANSWER 21 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 1996:564403 CAPLUS
DN 125:215364
TI Purification, N-terminal amino acid sequencing and antifungal activity of chitinases from pepper stems treated with mercuric chloride
AU Kim, Young Jin; Hwang, Byung Kook
CS Department Agricultural Biology, Korea University, Seoul, 136-701, S. Korea
SO Physiol. Mol. Plant Pathol. (1996), 48(6), 417-432
CODEN: PMPPEZ; ISSN: 0885-5765
DT Journal
LA English
AB Different isoforms of chitinases were purified from pepper (Capsicum annuum L. cv. Hanbyul) stems treated with mercuric chloride. The acidic isoform a1 (69 kDa, pI 5.sum.0), basic isoforms b1 (32 kDa, pI 9.sum.0) and b2 (22 kDa, pI 9.sum.1) were purified by chitin-affinity chromatog., with subsequent electroelution from nondenaturing PAGE (PAGE) gels. The acidic isoform a1 has chitin-binding properties, but no antifungal activity. The basic isoforms b1 and b2 contain high ratios of cysteine and glycine at the N-terminal ***chitin*** - ***binding*** ***domain*** , exhibit chitinase activity, and show antifungal activities against Colletotrichum gloeosporioides, Fusarium oxysporum f.sp. cucumerinum, Magnaporthe grisea, and Trichoderma viride in vitro. Moreover, their antifungal activity shows a high degree of specificity to filamentous fungi. The chitinases b1 and b2 show a high sequence identity in their N-terminal residues with those from wheat, tobacco, potato, rice and Arabidopsis thaliana. None of the purified isoforms of chitinases inhibited hyphal growth of the Oomycete fungus which lacks chitin Phytophthora capsici. In contrast, zoospore germination and germ tube elongation of P. capsici were effectively inhibited by treatment with b1 and b2.

L18 ANSWER 22 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 1995:291472 CAPLUS
DN 122:50890
TI The chitinolytic system of Streptomyces olivaceoviridis
AU Schrempf, H.; Schnellmann, J.; Zeltins, A.; Blaak, H.
CS Department Biology, University Osnabruck, Osnabrueck, 49069, Germany
SO Meded. - Fac. Landbouwkd. Toegepaste Biol. Wet. (Univ. Gent) (1994), 59(4B), 2443-51
CODEN: MFLBER
DT Journal
LA English
AB Streptomyces olivaceoviridis produces several chitinases and degrades chitin efficiently. Shotgun cloning of partially Sau3A cleaved DNA using the multicopy vector pIJ702 and Streptomyces lividans 66 as host resulted in the identification of the plasmid pCHI01. In the presence of chitin as sole carbon source, transformants of Streptomyces lividans 66 carrying pCHI01 secreted large quantities of a chitin-inducible exochitinase of 59 kDa which was found to bind strongly to the cryst. medium. In the course of cultivation, the 59 kDa enzyme was processed proteolytically to a truncated 47 kDa, still active enzyme, which was then released to the culture filtrate. The purified 47 kDa enzyme has an isoelec. point of 4.0, shows optimal activity at pH 7.3 and 55.degree.C and is competitively inhibited by the pseudosugar allosamidin. The enzyme was identified as an exochitinase since it generates exclusively chitobiose from chitotetraose, chitohexaose, and colloidal high-mol. mass chitin. Sequence anal. of a reading frame of 1794 base pairs, comparison of the deduced amino acid sequence, and biochem. studies of the mature protein (59 kDa) and the proteolytically processed form (47 kDa) allowed the identification of the C-terminal catalytic domain, one central region with significant similarity to the type III module of fibronectin, and one N-terminal ***chitin*** - ***binding*** ***domain*** (12 kDa). During cultivation in the presence of chitin as sole carbon source, Streptomyces olivaceoviridis secretes several different chitinases and a 18.7 kDa chitin-binding protein (CHB1). If grown in the presence of crab chitin, transformants of Streptomyces lividans 66 harboring the cloned chb1 gene on a multicopy vector overproduced large quantities of the 18.7 kDa ***protein*** which was ***purified*** to homogeneity. Biochem. studies and immunofluorescence microscopy revealed that the CHB1 protein binds strongly to .alpha.-chitin from crab shell or fungi, but neither to .beta.-chitin nor to various cellulose types. A reading frame of 606 bp was shown to encode the CHB1 protein. Amino acid sequence comparisons and biochem. studies allowed the identification of amino acids which appear to be involved in the interaction with chitin. The role of this novel lectin-like protein is at present being investigated.

L18 ANSWER 23 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
11
AN 1994:133439 BIOSIS
DN PREV199497146439
TI A novel pathogen- and wound-inducible tobacco (Nicotiana tabacum) protein with antifungal activity.
AU Ponstein, Anne S. (1); Bres-Vloemans, Sandra A.; Sela-Buurlage, Marianne B.; Van Den Elzen, Peter J. M.; Melchers, Leo S.; Cornelissen, Ben J. C.
CS (1) MOGEN Int. NV, 2333 CB Leiden Netherlands
SO Plant Physiology (Rockville), (1994) Vol. 104, No. 1, pp. 109-118.
ISSN: 0032-0889.
DT Article
LA English
AB A novel pathogen- and wound-inducible antifungal protein of 20 kD was purified from tobacco (Nicotiana tabacum) Samsun NN leaves inoculated with tobacco mosaic virus (TMV). The ***protein*** , designated CBP20, was ***purified*** by chitin-affinity chromatography and gel filtration. In vitro assays demonstrated that CBP20 exhibits antifungal activity toward Trichoderma viride and Fusarium solani by causing lysis of the germ tubes and/or growth inhibition. In addition it was shown that CBP20 acts synergistically with a tobacco class I chitinase against F. solani and with a tobacco class 1 beta-1,3-glucanase against F. solani and Alternaria radicina. Analysis of the protein and corresponding cDNAs revealed that CBP20 contains an N-terminal ***chitin*** - ***binding*** ***domain*** that is present also in the class I chitinases of tobacco, the putative wound-induced (WIN) proteins of potato, WIN1 and WIN2, and several plant lectins. The C-terminal domain of CBP20 showed high identity with tobacco pathogenesis-related (PR) proteins, PR-4a and PR-4b, tomato PR-P2, and potato WIN1 and WIN2. CBP20 is synthesized as a preproprotein, which is processed into the mature protein by the removal of an N-terminal signal peptide and a C-terminal propeptide, most likely involved in the vacuolar targeting of the protein. The intracellular localization of CBP20 and its induction upon TMV infection and wounding indicate that CBP20 is the first class I PR-4 type ***protein*** ***purified*** .

L18 ANSWER 24 OF 24 CAPLUS COPYRIGHT 2002 ACS
AN 1994:126945 CAPLUS
DN 120:126945
TI Immobilization and purification of fusion proteins using chitin-binding ability
IN Kuranda, Michael J.
PA Massachusetts Institute of Technology, USA
SO U.S., 18 pp.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 5258502	A	19931102	US 1989-303827	19890130

AB The noncatalytic ***chitin*** - ***binding*** ***domain*** of yeast endochitinase is incorporated into fusion proteins for ease of ***purifn*** . of the ***protein*** . The fusion ***protein*** is rapidly ***purified*** by affinity chromatog. on chitin. A chimeric gene encoding a fusion protein of yeast invertase and the ***chitin*** - ***binding*** ***domain*** and the chitinase signal peptide was constructed by std. methods and expressed in a chitinase-deficient yeast using the endochitinase gene promoter. The protein was recovered from the medium by adsorption onto chitin. The protein showed the mol. wt. and properties expected.

=> FIL STNGUIDE

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FULL ESTIMATED COST		104.89	138.42

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FILE CONTAINS CURRENT INFORMATION.
LAST RELOADED: Apr 12, 2002 (20020412/UP).

=> FIL BIOSIS MEDLINE EMBASE

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FULL ESTIMATED COST		0.18	138.60

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	ENTRY	SINCE FILE SESSION	TOTAL SESSION
CA SUBSCRIBER PRICE		0.00	-6.20

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=> d his

(FILE 'HOME' ENTERED AT 13:24:48 ON 19 APR 2002)

FILE 'BIOSIS, MEDLINE, EMBASE' ENTERED AT 13:26:10 ON 19 APR 2002
L1 364 S ANTIBODY PURIFICATION
L2 279 S L1 AND PY<1999

FILE 'STNGUIDE' ENTERED AT 13:36:11 ON 19 APR 2002

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 13:45:32 ON 19 APR 2002
L3 1092 S CELLULOSE BINDING DOMAIN
L4 2 S L3 AND L1
L5 1 DUP REM L4 (1 DUPLICATE REMOVED)
L6 13521 S ANTIBODY (3A) PURIF?
L7 3 S L6 AND L3
L8 1 DUP REM L7 (2 DUPLICATES REMOVED)
L9 266 S CHITIN BINDING DOMAIN
L10 0 S L9 AND L6
L11 2656 S PROTEIN L
L12 1 S L11 AND L3
L13 0 S L9 AND L11
L14 96199 S PROTEIN (3A) PURIF?
L15 95 S L3 AND L14
L16 56 DUP REM L15 (39 DUPLICATES REMOVED)
L17 37 S L9 AND L14
L18 24 DUP REM L17 (13 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 14:05:32 ON 19 APR 2002

FILE 'BIOSIS, MEDLINE, EMBASE' ENTERED AT 14:07:03 ON 19 APR 2002

=> d bib abs l16 1-10
YOU HAVE REQUESTED DATA FROM FILE 'BIOSIS, EMBASE, CAPLUS' - CONTINUE? (Y)/N:y

L16 ANSWER 1 OF 56 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2002091691 EMBASE
TI Purification and characterization of new endo-1,4-.beta.-D-glucanases from Rhizopus oryzae.
AU Murashima K.; Nishimura T.; Nakamura Y.; Koga J.; Moriya T.; Sumida N.; Yaguchi T.; Kono T.
CS K. Murashima, Bio Science Laboratories, Meiji Seika Kaisha, Ltd., Saitama, Japan. Koichiro_murashima@meiji.co.jp
SO Enzyme and Microbial Technology, (13 Mar 2002) 30/3 (319-326).
Refs: 25
ISSN: 0141-0229 CODEN: EMTED2
PUI S 0141-0229(01)00513-0
CY United States
DT Journal; Conference Article
FS 004 Microbiology
LA English
SL English
AB New extracellular endoglucanases, designated RCE1 and RCE2, produced by Rhizopus oryzae isolated from the soil, were purified to apparent homogeneity from the culture supernatant. The molecular mass of RCE1 and that of RCE2 were found to be 41 kDa and 61 kDa, respectively. The N-terminal amino acid sequences of RCE1 and RCE2 showed high homology with those of the family I cellulose-binding domains. Internal amino acid sequences of RCE1 and RCE2 showed homology with that of the catalytic domain of EGV from Humicola insolens belonging to family 45 endoglucanase. The cellooligosaccharide hydrolysis patterns of RCE1 and RCE2 were similar to that of EGV from H. insolens. These results indicate that RCE1 and RCE2 are family 45 endoglucanases having a ***cellulose*** ***binding*** ***domain*** at their N-terminus. RCE1 and RCE2 hydrolyzed carboxymethylcellulose (CMC), insoluble cellooligosaccharide (G33), cellohexaose, and cellopenpaose, but not Avicel, xylan, galactan, arabinan, mannan, or laminarin. The CMCase activity of both enzymes was inhibited by Cu(2+), Zn(2+), Co(2+), and Pb(2+). The optimum pH for the CMCase activity of both enzymes was found to be between pH value 5.0 and 6.0, and the optimum temperature was 55.degree.C, the lowest among the family 45 endoglucanases. These results indicate that RCE1 and RCE2 represent a new type of endoglucanases having the lowest optimum temperature among the family 45 endoglucanases. .COPYRGT. 2002 Elsevier Science Inc. All rights reserved.

L16 ANSWER 2 OF 56 CAPLUS COPYRIGHT 2002 ACS
AN 2002:178717 CAPLUS
TI Effects of a ***cellulose*** ***binding*** ***domain*** on deinking of recycled mixed office paper
AU Li, Kaichang; Xu, Xia
CS Department of Wood Science and Engineering, Oregon State University, Corvallis, OR, 97331, USA

SO Progress in Paper Recycling (2002), 11(2), 9-13
CODEN: PPREFY; ISSN: 1061-1452
PB Doshi & Associates Inc.
DT Journal
LA English
AB A fusion protein contg. a ***cellulose*** ***binding*** ***domain*** (CBD) from Cellulomonas fimi endoglucanase A was prepd. and purified. The ***purified*** CBD ***protein*** was used in the deinking of a mixed office paper (MOP). The deinking process included the repulping of the MOP, incubation of MOP pulp slurry with the CBD protein, and a flotation. It was found that the incubation of the pulp slurry with the CBD greatly increased total dirt count and residual ink area. Direct addn. of the CBD to the flotation stage also increased dirt count and residual ink area significantly. The overall redn. of the deinking efficiency by the CBD was due to a decrease in the efficiency of ink removal in the flotation stage.
RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 3 OF 56 CAPLUS COPYRIGHT 2002 ACS
AN 2001:283713 CAPLUS
DN 134:291115
TI Manufacture of proteins in milk as complexes with binding partners and affinity purification of the complex
IN Meade, Harry; Fulton, Scott P.; Echelard, Yann
PA Genzyme Transgenics Corporation, USA
SO PCT Int. Appl., 43 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2001026455	A1	20010419	WO 2000-US28589	20001016

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 1999-159748P P 19991014
US 2000-204662P P 20000517
AB The invention provides systems and methods for the prodn. and purifn. of target mols. present in biol. systems. The systems and methods according to the invention utilize transgenic expression of multivalent binding polypeptides, as affinity media, to purify such target mols. The transgenic multivalent binding polypeptides bind both the target mols., e.g., a bindable epitope of a target mol., and a matrix. Specifically, proteins are manufd. in the mammary gland of a transgenic mammal and secreted into the milk. A natural binding partner of the protein is also manufd. in milk where it forms a stabilizing complex with the target protein. If the binding partner is labeled with an affinity label, the complex can quickly purified by affinity chromatog. Alternatively, the protein can be manufd. labeled with a cleavable epitope. After capture with the cognate antibody, the protein can be released by cleaving it from the epitope.
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 4 OF 56 CAPLUS COPYRIGHT 2002 ACS
AN 2001:43459 CAPLUS
DN 134:114914
TI Purification of a polypeptide compound having a polysaccharide binding domain by affinity phase separation
IN Haynes, Charles A.; Tomme, Peter; Kilburn, Douglas G.
PA University of British Columbia, Can.
SO U.S., 44 pp., Cont.-in-part of U.S. Ser. No. 249,037.
CODEN: USXXAM
DT Patent
LA English
FAN.CNT 6

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 6174700	B1	20010116	US 1995-505860	19950724
US 5137819	A	19920811	US 1988-216794	19880708
US 5202247	A	19930413	US 1990-603987	19901025
US 5340731	A	19940823	US 1992-865095	19920408
US 5928917	A	19990727	US 1994-249037	19940524
CA 2226785	AA	19970417	CA 1996-2226785	19960724
WO 9713841	A1	19970417	WO 1996-US12282	19960724

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA

AU 9666805	A1	19970430	AU 1996-66805	19960724
AU 718247	B2	20000413		
EP 842264	A1	19980520	EP 1996-926776	19960724

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

CN 1193995	A	19980923	CN 1996-196571	19960724
BR 9609742	A	19990302	BR 1996-9742	19960724
JP 11509738	T2	19990831	JP 1996-511489	19960724
US 6048715	A	20000411	US 1996-685808	19960724

PRAI US 1988-216794 A3 19880708
US 1990-603987 A2 19901025
US 1992-865095 A1 19920408
US 1994-249037 A2 19940524
US 1995-505860 A 19950724
WO 1996-US12282 W 19960724
AB A compd. having a polysaccharide-binding domain such as contained by a cellulose and essentially lacking in polysaccharidase activity is purified from other ingredients in a mixt. using an affinity partition system. A mixt. contg. the compd. is contacted with a system contg. as a 1st phase an aq. soln. of oligosaccharide polymer such as cellulose and as a 2nd phase a soln. of a polymer such as a poly(ethylene glycol)-poly(propylene glycol) copolymer. The compd. partitions into the 1st phase and binds to the oligosaccharide polymer, preferably with a Ka of 103-107, to form a complex. The complex is collected, and the compd. is dissocd. from the oligosaccharide polymer. The compd. may be formed of a non-peptide chem. moiety or a peptide moiety linked to a polypeptide having the polysaccharide-binding domain. The compd. may also be a fusion polypeptide contg. the polysaccharide-binding domain linked through a protease recognition sequence to a macromol. such as an enzyme, a hormone, or an antibody. The macromol. can be removed by using a protease to cleave the recognition sequence. Another partition system contains the oligosaccharide polymer and a phase sepn. inducing agent such as a sulfate or citrate salt that induces sepn. to produce different phases.
RE.CNT 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 5 OF 56 CAPLUS COPYRIGHT 2002 ACS
AN 2001:142087 CAPLUS
DN 134:190009
TI Thermostable chitinase from hyperthermophilic archaeon Pyrococcus kodakaraensis KOD1 and mutants
IN Imanaka, Tadayuki
PA Japan
SO Jpn. Kokai Tokkyo Koho, 19 pp.
CODEN: JKXXAF
DT Patent
LA Japanese
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI JP 2001054381	A2	20010227	JP 1999-229517	19990813

AB A highly heat-resistant chitinase from Pyrococcus kodakaraensis KOD1 and its mutants, recombinant expression, are disclosed. We have found that the hyperthermophilic archaeon Pyrococcus kodakaraensis KOD1 produces an extracellular chitinase. The gene encoding the chitinase (chiA) was cloned and sequenced. The chiA gene was found to be composed of 3,645 nucleotides, encoding a protein (1,215 amino acids) with a mol. mass of 134,259 Da, which is the largest among known chitinases. Sequence anal. indicates that ChiA is divided into two distinct regions with resp. active sites. The N-terminal and C-terminal regions show sequence similarity with chitinase A1 from Bacillus circulans WL-12 and chitinase from Streptomyces erythraeus (ATCC 11635), resp. Furthermore, ChiA possesses unique chitin binding domains (CBDs) (CBD1, CBD2, and CBD3) which show sequence similarity with cellulose binding domains of various cellulases. CBD1 was classified into the group of family V type cellulose binding domains. In contrast, CBD2 and CBD3 were classified into that of the family II type. ChiA was expressed in Escherichia coli cells, and the recombinant ***protein*** was ***purified*** to homogeneity. The optimal temp. and pH for chitinase activity were found to be 85.degree. and 5.0, resp. Results of thin-layer chromatog. anal. and activity measurements with fluorescent substrates suggest that the enzyme is an endo-type enzyme which produces a chitobiose as a major end product. Various deletion mutants were constructed, and analyses of their enzyme characteristics revealed that both the N-terminal and C-terminal halves are independently functional as chitinases and that CBDs play an important role in insol. chitin binding and hydrolysis. Deletion mutants which contain the C-terminal half showed higher thermostability than did N-terminal-half mutants and wild-type ChiA. Some of the mutants retained 90% of their activity even after 100.degree.C treatment for 3 h. The highly thermostable chitinase mutants either contained Strepymyces erythraeus chitinase homol. domain or lacked Bacillus circulans chitinase homol. domain. The enzyme also contained Butyrivibrio fibrisolvens cellulase cellulose binding domains (CBD).

L16 ANSWER 6 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE
1
AN 2001:371809 BIOSIS
DN PREV200100371809
TI Cloning and sequencing of a molluscan endo-beta-1,4-glucanase gene from the blue mussel, Mytilus edulis.
AU Xu, Bingze; Janson, Jan-Christer; Sellos, Daniel (1)
CS (1) Station de Biologie Marine, Museum National d'Histoire Naturelle,

29182, Concarneau Cedex: Sellos@mnhn.fr France
SO European Journal of Biochemistry, (July, 2001) Vol. 268, No. 13, pp. 3718-3727. print.
ISSN: 0014-2956.
DT Article
LA English
SL English
AB Using polymerase chain reaction, cloning and sequencing techniques, a complementary DNA encoding a low molecular mass cellulase (endo-1,4-beta-D-glucanase, EC 3.2.1.4) has been identified in the digestive gland of the marine mussel, Mytilus edulis. It contains a 5' untranslated region, a 633-nucleotide ORF encoding a 211 amino-acid protein, including a 17 amino-acid signal peptide and a complete 3' untranslated region. At the C-terminal end of the ***purified*** mature ***protein*** , a 13 amino-acid peptide is lacking in comparison to the protein sequence deduced from the ORF. This peptide is probably removed as a consequence of post-translational amidation of the C-terminal glutamine. The endoglucanase genes have been isolated and sequenced from both Swedish and French mussels. The coding parts of these two sequences are identical. Both genes contain two introns, the positions of which are conserved. However the length of the introns are different due to base substitutions, insertions or deletions showing the existence of interspecies length polymorphism. The percentage of similarity for the introns of the two gene sequences is 96.9%. This is the first time a molluscan cellulase is characterized at DNA level. Amino acid sequence-based classification has revealed that the enzyme belongs to the glycosyl hydrolase family 45 (B. Henrissat (Centre de Recherches sur les Macromolecules Vegetales, CNRS, Joseph Fourier Universite, Grenoble, France), personal communication). There is no ***cellulose*** ***binding*** ***domain*** associated with the sequence.

L16 ANSWER 7 OF 56 CAPLUS COPYRIGHT 2002 ACS
AN 2000:720308 CAPLUS
DN 133:280644
TI Process for partitioning of proteins
IN Penttila, Merja; Nakari-Setala, Tiina; Fagerstrom, Richard; Selber, Klaus; Kula, Maria-regina; Linder, Markus; Tjerneld, Folke
PA Valtion Teknillinen Tutkimuskeskus, Finland
SO PCT Int. Appl., 109 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000058342	A1	20001005	WO 2000-FI249	20000324

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

EP 1163260	A1	20011219	EP 2000-914217	20000324

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO

NO 2001004534	A	20011126	NO 2001-4534	20010918
PRAI FI 1999-667	A	19990325		
FI 1999-1782	A	19990820		
WO 2000-FI249	W	20000324		

AB The present invention provides a method for isolation and purifn. of proteins in aq. two-phase systems (ATPS). Specifically the invention provides processes for partitioning of proteins in ATPS by fusing the protein of interest to a targeting protein which has the ability of carrying the desired protein into one of the phases. Thus, the core of endoglucanase I (EGI)from Trichoderma reesii was produced in fed-batch fermns. as a fusion protein with the small protein hydrophobin I (HFBI). The fermn. broth was clarified by centrifugation, and the EGI-HFBI fusion protein was sepd. from the supernatant by ATPS using 2% (wt./wt.) of the detergent C12-18-EO5. The ***purified*** fusion ***protein*** enriched in the top detergent phase was then removed by extn. with isobutanol.
RE.CNT 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 8 OF 56 CAPLUS COPYRIGHT 2002 ACS
AN 2000:666862 CAPLUS
DN 133:248956
TI Cloning, expression, purifn. and characterization of a pectate lyase from Bacillus and its use in laundering and textile processing
IN Bjornvad, Mads Eskelund; Andersen, Jens Tonne; Schnorr, Kirk; Schulein, Martin; Kongsbak, Lars
PA Novo Nordisk A/s, Den.
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000055309	A1	20000921	WO 2000-DK111	20000315

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI DK 1999-367	A	19990316		

AB Cloning, expression, purifn. and characterization of a pectate lyase from Bacillus sp. belonging to a novel family of polysaccharide lyases is disclosed. Nucleotide sequence of the pectate lyase gene and amino acid sequence of the encoded enzyme are reported. This novel pectate lyase of Bacillus has good performance in industrial processes under neutral or alk. conditions such as laundering and textile processing.
RE.CNT 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 9 OF 56 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2000294130 EMBASE
TI A scaffoldin of the Bacteroides cellulosolvens cellulosome that contains 11 type II cohesins.
AU Ding S.-Y.; Bayer E.A.; Steiner D.; Shoham Y.; Lamed R.
CS E.A. Bayer, Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel. bfbayer@wicc.weizmann.ac.il
SO Journal of Bacteriology, (2000) 182/17 (4915-4925).
Refs: 40
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB A cellulosomal scaffoldin gene, termed cipBc, was identified and sequenced from the mesophilic cellulolytic anaerobe Bacteroides cellulosolvens. The gene encodes a 2,292-residue polypeptide (excluding the signal sequence) with a calculated molecular weight of 242,437. CipBc contains an N-terminal signal peptide, 11 type II cohesin domains, an internal family III ***cellulose*** - ***binding*** ***domain*** (CBD), and a C-terminal dockerin domain. Its CBD belongs to family IIIb, like that of CipV from Acetivibrio cellulolyticus but unlike the family IIIa CBDs of other clostridial scaffoldins. In contrast to all other scaffoldins thus far described, CipBc lacks a hydrophilic domain or domain X of unknown function. The singularity of CipBc, however, lies in its numerous type II cohesin domains, all of which are very similar in sequence. One of the latter cohesin domains was expressed, and the expressed protein interacted selectively with cellulosomal enzymes, one of which was identified as a family 48 glycosyl hydrolase on the basis of partial sequence alignment. By definition, the dockerins, carried by the cellulosomal enzymes of this species, would be considered to be type II. This is the first example of authentic type II cohesins that are confirmed components of a cellulosomal scaffoldin subunit rather than a cell surface anchoring component. The results attest to the emerging diversity of cellulosomes and their component sequences in nature.

L16 ANSWER 10 OF 56 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 2000394266 EMBASE
TI Alpha-amylase inhibitors selected from a combinatorial library of a ***cellulose*** ***binding*** ***domain*** scaffold.
AU Lehtio J.; Teeri T.T.; Nygren P.-A.
CS P.-A. Nygren, Department of Biotechnology, Royal Institute of Technology, SE-100 44 Stockholm, Sweden. perake@biochem.kth.se
SO Proteins: Structure, Function and Genetics, (15 Nov 2000) 41/3 (316-322).
Refs: 48
ISSN: 0887-3585 CODEN: PSFGEY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB A disulfide bridge-constrained ***cellulose*** ***binding*** ***domain*** (CBD(WT)) derived from the cellobiohydrolase Cel7A from Trichoderma reesei has been investigated for use in scaffold engineering to obtain novel binding proteins. The gene encoding the wild-type 36 aa CBD(WT) domain was first inserted into a phagemid vector and shown to be functionally displayed on M13 filamentous phage as a protein III fusion protein with retained cellulose binding activity. A combinatorial library comprising 46 million variants of the CBD domain was constructed through randomization of 11 positions located at the domain surface and distributed over three separate .beta.-sheets of the domain. Using the enzyme porcine alpha-amylase (PPA) as target in biopannings, two CBD variants showing selective binding to the enzyme were characterized. Reduction and iodoacetamide blocking of cysteine residues in selected CBD variants resulted in a loss of binding activity, indicating a conformation dependent binding. Interestingly, further studies showed that the selected CBD variants were capable of competing with the binding of the amylase inhibitor acarbose to the enzyme. In addition, the enzyme activity could be partially inhibited by addition of soluble protein, suggesting that the selected CBD variants bind to the active site of the enzyme. (C) 2000 Wiley-Liss, Inc.

=> d his

(FILE 'HOME' ENTERED AT 13:24:48 ON 19 APR 2002)

FILE 'BIOSIS, MEDLINE, EMBASE' ENTERED AT 13:26:10 ON 19 APR 2002
L1 364 S ANTIBODY PURIFICATION
L2 279 S L1 AND PY<1999

FILE 'STNGUIDE' ENTERED AT 13:36:11 ON 19 APR 2002

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 13:45:32 ON 19 APR 2002
L3 1092 S CELLULOSE BINDING DOMAIN
L4 2 S L3 AND L1
L5 1 DUP REM L4 (1 DUPLICATE REMOVED)
L6 13521 S ANTIBODY (3A) PURIF?
L7 3 S L6 AND L3
L8 1 DUP REM L7 (2 DUPLICATES REMOVED)
L9 266 S CHITIN BINDING DOMAIN
L10 0 S L9 AND L6
L11 2656 S PROTEIN L
L12 1 S L11 AND L3
L13 0 S L9 AND L11
L14 96199 S PROTEIN (3A) PURIF?
L15 95 S L3 AND L14
L16 56 DUP REM L15 (39 DUPLICATES REMOVED)
L17 37 S L9 AND L14
L18 24 DUP REM L17 (13 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 14:05:32 ON 19 APR 2002

FILE 'BIOSIS, MEDLINE, EMBASE' ENTERED AT 14:07:03 ON 19 APR 2002

FILE 'BIOSIS, EMBASE, CAPLUS' ENTERED AT 14:07:30 ON 19 APR 2002

FILE 'BIOSIS, MEDLINE, EMBASE' ENTERED AT 14:07:32 ON 19 APR 2002

=> s l16 and py<1999
1 FILES SEARCHED...
L19 28 L16 AND PY<1999

=> d bib abs 1-
YOU HAVE REQUESTED DATA FROM 28 ANSWERS - CONTINUE? Y/(N):y

L19 ANSWER 1 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:153763 BIOSIS
DN PREV199900153763
TI ***Purification*** of a fusion ***protein*** using the family VI ***cellulose*** - ***binding*** ***domain*** of Clostridium stercorarium XynA.
AU Sakka, Kazuo (1); Karita, Shuichi; Kimura, Tetsuya (1); Ohmiya, Kunio (1)
CS (1) Fac. Bioresources, Mie Univ., Tsu 514 Japan
SO Laskin, A. I. [Editor]; Li, G.-X. [Editor]; Yu, Y.-T. [Editor]. Annals of the New York Academy of Sciences, (***Dec. 13, 1998***) Vol. 864, pp. 485-488. Annals of the New York Academy of Sciences; Enzyme engineering XIV.
Publisher: New York Academy of Sciences 2 East 63rd Street, New York, New York 10021, USA.
Meeting Info.: Fourteenth International Enzyme Engineering Conference Beijing, China October 12-17, 1997 Engineering Foundation, New York . ISSN: 0077-8923. ISBN: 1-57331-149-9 (cloth), 1-57331-150-2 (paper).
DT Book; Conference
LA English

L19 ANSWER 2 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:48630 BIOSIS
DN PREV199900048630
TI Isolation of the gene and characterization of the enzymatic properties of a major exoglucanase of Humicola grisea without a ***cellulose*** - ***binding*** ***domain*** .
AU Takashima, Shou; Iikura, Hiroshi; Nakamura, Akira; Hidaka, Makoto; Masaki, Haruhiko; Uozumi, Takeshi
CS Dep. Biotechnol., Grad. Sch. Agric. Life Sci., Univ. Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657 Japan
SO Journal of Biochemistry (Tokyo), (***Oct., 1998***) Vol. 124, No. 4, pp. 717-725.
ISSN: 0021-924X.
DT Article
LA English
AB An exoglucanase gene was cloned from a cellulolytic fungus, Humicola grisea. DNA sequencing of this gene, designated as exo1, revealed that it contained four introns in the coding region. The deduced amino acid sequence of EXO1 was 451 amino acids in length and showed 57.7% identity with that of H. grisea cellobiohydrolase 1 (CBH1), but lacked the typical domain structures of a ***cellulose*** - ***binding*** ***domain*** and a hinge region. Transcriptional analysis of the exo1 and cbh1 genes showed that the expression of these genes was induced by Avicel, and repressed in the presence of glucose. The exo1 gene was expressed in Aspergillus oryzae, and the recombinant EXO1 ***protein*** was ***purified*** . EXO1 and CBH1 produced by A. oryzae showed relatively higher activity toward Avicel, but showed much lower activity toward carboxymethyl cellulose (CMC) and p-nitrophenyl-beta-D-cellobioside (PNPC), than H. grisea endoglucanase 1 (EGL1). The addition of a ***cellulose*** - ***binding*** ***domain*** and a hinge region to EXO1 caused decreases in its enzymatic activities as well as the deletion of the ***cellulose*** - ***binding*** ***domain*** from CBH1. EXO1 showed relatively weak or no synergistic activity toward Avicel with H. grisea endoglucanases, but showed a significant level of apparent synergism with H. grisea CBH1 and Trichoderma reesei EGLI. CBH1 showed a significant level of apparent endo-exo synergism with H. grisea and T. reesei endoglucanases. H. grisea has at least two different types of major exoglucanase components and shows strong cellulolytic activity through synergism with cellulase components including EXO1 and CBH1.

L19 ANSWER 3 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:12632 BIOSIS
DN PREV199900012632
TI Production and purification of a recombinant human hsp60 epitope using the ***cellulose*** - ***binding*** ***domain*** in Escherichia coli.
AU Shpigel, Etai (1); Elias, Dana; Cohen, Irun R.; Shoseyov, Oded
CS (1) Kennedy Leigh Cent. Hortic. Res., Fac. Agric., Hebrew Univ. Jerusalem, P.O. Box 12, Rehovot 76100 Israel
SO Protein Expression and Purification, (***Nov., 1998***) Vol. 14, No. 2, pp. 185-191.
ISSN: 1046-5928.
DT Article
LA English
AB The heat shock protein hsp60 plays a functional role in insulin-dependent diabetes mellitus. The hsp60 epitope p277 (aa 437-aa 460) is effective in vaccinating mice against diabetes. A synthetic peptide gene (p277) that encodes the human hsp60 epitope was cloned to the 3' end of the ***cellulose*** - ***binding*** ***domain*** gene (cbd). CBD-p277 was overexpressed in Escherichia coli and purified on a cellulose column. A methionine at the C-terminal end of CBD enabled CNBr cleavage be. tween CBD and p277. After CNBr cleavage, free CBD and residual uncleaved CBD-p277 were recovered by cellulose chromatography. The p277 peptide was further purified on a RPC-FPLC column. The molecular weight of the recombinant peptide was confirmed by electrospray mass spectrometry. The recombinant peptide was found to be biologically active in assays involving clone C9 T-cell proliferation, lymph-node cell proliferation, and antibody production. Thus the use of CBD as an affinity tag and the utilization of affordable cellulose matrices offers an attractive method for the production and purification of recombinant peptides.

L19 ANSWER 4 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:226167 BIOSIS
DN PREV199800226167
TI Roles of the catalytic domain and two cellulose binding domains of Thermomonospora fusca E4 in cellulose hydrolysis.
AU Irwin, Diana; Shin, Dong-Hoon; Zhang, Sheng; Barr, Brian K.; Sakon, Joshua; Karplus, P. Andrew; Wilson, David B. (1)
CS (1) BMCB, Biotechnol. Build., Cornell Univ., Ithaca, NY 14853 USA
SO Journal of Bacteriology, (***April, 1998***) Vol. 180, No. 7, pp. 1709-1714.
ISSN: 0021-9193.
DT Article
LA English
AB Thermomonospora fusca E4 is an unusual 90.4-kDa endocellulase comprised of a catalytic domain (CD), an internal family IIIc ***cellulose*** ***binding*** ***domain*** (CBD), a fibronectinlike domain, and a family II CBD. Constructs containing the CD alone (E4-51), the CD plus the family IIIc CBD (E4-68), and the CD plus the fibronectinlike domain plus the family II CBD (E4-74) were made by using recombinant DNA techniques. The activities of each ***purified*** ***protein*** on bacterial microcrystalline cellulose (BMCC), filter paper, swollen cellulose, and carboxymethyl cellulose were measured. Only the whole enzyme, E4-90, could reach the target digestion of 4.5% on filter paper. Removal of the internal family IIIc CBD (E4-51 and E4-74) decreased activity markedly on every substrate. E4-74 did bind to BMCC but had almost no hydrolytic activity, while E4-68 retained 32% of the activity on BMCC even though it did not bind. A low-activity mutant of one of the catalytic bases, E4-68 (Asp55Cys), did bind to BMCC, although E4-51 (Asp55Cys) did not. The ratios of soluble to insoluble reducing sugar produced after filter paper hydrolysis by E4-90, E4-68, E4-74, and E4-51 were 6.9, 3.5, 1.3, and 0.6, respectively, indicating that the family IIIc CBD is important for E4 processivity.

L19 ANSWER 5 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:83581 BIOSIS
DN PREV199800083581
TI Purification of the Ruminococcus albus endoglucanase IV using a ***cellulose*** - ***binding*** ***domain*** as an affinity tag.
AU Karita, Shuichi (1); Kimura, Tetsuya; Sakka, Kazuo; Ohmiya, Kunio
CS (1) Cent. Mol. Biol. Genet., Mie Univ., Tsu 514 Japan
SO Journal of Fermentation and Bioengineering, (1997) Vol. 84, No. 4, pp. 354-357.
ISSN: 0922-338X.
DT Article
LA English
AB The gene encoding the single ***cellulose*** - ***binding***

domain II (CBD II) of Clostridium stercorarium xylanase A was fused to the egIV gene encoding endoglucanase IV (EGIV) from Ruminococcus albus. The fusion protein (EGIV + CBDII) expressed in Escherichia coli can be readily purified from the cell-free extract of E. coli in a single step using the affinity of CBD to cellulose. The purified enzyme was cleaved into two moieties, i.e. the catalytic domain and CBD, at a specific site in the linker region by partial digestion with trypsin at 4degree C. This result indicates that this CBD belonging to family VI of CBD families can be used as an affinity tag for ***purification*** of the recombinant ***protein*** .

L19 ANSWER 6 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:4357 BIOSIS
DN PREV199800004357
TI CelG from Clostridium cellulolyticum: A multidomain endoglucanase acting efficiently on crystalline cellulose.
AU Gal, Laurent; Gaudin, Christian (1); Belaich, Anne; Pages, Sandrine; Tardif, Chantal; Belaich, Jean-Pierre
CS (1) Lab. Bioenerget. Ingenierie Proteines, CNRS, 31, Chemin Joseph Aiguier, 13402 Marseille Cedex 20 France
SO Journal of Bacteriology, (***Nov., 1997***) Vol. 179, No. 21, pp. 6595-6601.
ISSN: 0021-9193.
DT Article
LA English
AB The gene coding for CelG, a family 9 cellulase from Clostridium cellulolyticum, was cloned and overexpressed in Escherichia coli. Four different forms of the ***protein*** were genetically engineered, ***purified*** , and studied: CelGL (the entire form of CelG), CelGcat1 (the catalytic domain of CelG alone), CelGcat2 (CelGcat1 plus 91 amino acids at the beginning of the ***cellulose*** ***binding*** ***domain*** (CBD)), and GST-CBDCelG (the CBD of CelG fused to glutathione S-transferase). The biochemical properties of CelG were compared with those of CelA, an endoglucanase from C. cellulolyticum which was previously studied. CelG, like CelA, was found to have an endo cutting mode of activity on carboxymethyl cellulose (CMC) but exhibited greater activity on crystalline substrates (bacterial microcrystalline cellulose and Avicel) than CelA. As observed with CelA, the presence of the nonhydrolytic miniscaffolding protein (miniCipC1) enhanced the activity of CelG on phosphoric acid swollen cellulose (PASC), but to a lesser extent. The absence of the CBD led to the complete inactivation of the enzyme. The abilities of CelG and GST-CBDCelG to bind various substrates were also studied. Although the entire enzyme is able to bind to crystalline cellulose at a limited number of sites, the chimeric protein GST-CBDCelG does not bind to either of the tested substrates (Avicel and PASC). The lack of independence between the two domains and the weak binding to cellulose suggest that this CBD-like domain may play a special role and be either directly or indirectly involved in the catalytic reaction.

L19 ANSWER 7 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1997:452682 BIOSIS
DN PREV199799751885
TI Cloning, expression in Streptomyces lividans and biochemical characterization of a thermostable endo-beta-1,4-xylanase of Thermomonospora alba ULJB1 with cellulose-binding ability.
AU Blanco, J.; Coque, J. J. R.; Velasco, J.; Martin, J. F. (1)
CS (1) Area Microbiol., Fac. Biol., Univ. Leon, 24071 Leon Spain
SO Applied Microbiology and Biotechnology, (1997) Vol. 48, No. 2, pp. 208-217.
ISSN: 0175-7598.
DT Article
LA English
AB Several thermophilic actinomycetes were isolated from urban solid waste. One of them, Thermomonospora alba ULJBI, showed a broad degradative activity on xylan, cellulose, starch and other polymers. Xylanase and cellulase activities were quantified and compared with those of Thermomonospora fusca. Genes encoding two different endo-beta-1,4-xylanases were cloned from T. alba ULJB 1. One of them, xylA, was sequenced, subcloned and overexpressed in Streptomyces lividans. It encodes a protein of 482 amino acids with a deduced molecular mass of 48 456 Da. The protein contains a 38 amino-acid leader peptide with six Arg+ residues in its amino-terminal end, a catalytic domain and a ***cellulose*** ***binding*** ***domain*** connected by a linker region rich in proline and glycine. The XylA ***protein*** was ***purified*** to near homogeneity from S. lividans/xylA cultures. Two forms of the extracellular xylanase, of 48 kDa and 38 kDa, were produced that differed in their cellulose binding ability. The 48-kDa protein showed a strong binding to cellulose whereas the 38-kDa form did not bind to this polymer, apparently because of the removal during processing of the ***cellulose*** - ***binding*** ***domain*** . Both forms were able to degrade xylans form different origins but not lichenam or carboxymethylcellulose. The major degradation product was xylobiose with traces of xylose. The xylanase activity was thermostable, showing a good activity up to 95 degree C, and had broad pH stability in the range from pH 4.0 to pH 10.0.

L19 ANSWER 8 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1997:179845 BIOSIS
DN PREV199799471558
TI Structure of the Clostridium stercorarium gene celY encoding the exo-1-4-beta-glucanase Avicelase II.
AU Bronnenmeier, Karin (1); Kundt, Kerstin; Riedel, Kathrin; Schwarz, Wolfgang H.; Staudenbauer, Walter L.
CS (1) Inst. Microbiol., Technical University Munich, Arcisstrasse 21, D-80290 Muenchen Germany
SO Microbiology (Reading), (1997) Vol. 143, No. 3, pp. 891-898.
ISSN: 1350-0872.
DT Article
LA English
AB The nucleotide sequence of the celY gene coding for the thermostable exo-1,4-beta-glucanase Avicelase II of Clostridium stercorarium was determined. The gene consists of an ORF of 2742 of Clostridium stercorarium was determined. The gene 742 bp which encodes a Preprotein of 914 amino acids with a molecular mass of 103 kDa. The signal-peptide cleavage site was identified by comparison with the N-terminal amino acid sequence of Avicelase 11 purified from C. stercorarium. The celY gene is located in close vicinity to the celZ gene coding for the endo-1,4-beta-glucanase Avicelase I. The CelY-encoding sequence was isolated from genomic DNA of C. stercorarium with the PCR technique- The recombinant enzyme produced in Escherichia coli as a LacZ'-CelY fusion ***protein*** could be ***purified*** Using a simple two-step procedure. The properties of CelY proved to be consistent with those of Avicelase II purified from C. stercorarium. Sequence comparison revealed that CelY consists of an N-terminal catalytic domain flanked by a domain of 95 amino acids with unknown function joined to a type III ***cellulose*** - ***binding*** ***domain*** . The catalytic domain belongs to the recently proposed family L of cellulases (family 48 of glycosyl hydrolases).

L19 ANSWER 9 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:468517 BIOSIS
DN PREV199699190873
TI Characterization of a double ***cellulose*** - ***binding*** ***domain*** . Synergistic high affinity binding to crystalline cellulose.
AU Linder, Markus (1); Salovuori, Irma; Ruohonen, Laura; Teeri, Tuula T.
CS (1) VTT/Biotechnol. Food Res., Box 1500, FIN-02044 VTT Finland
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 35, pp. 21268-21272.
ISSN: 0021-9258.
DT Article
LA English
AB Most cellulose-degrading enzymes have a two-domain structure that consists of a catalytic and a ***cellulose*** - ***binding*** ***domain*** (CBD) connected by a linker region. The linkage and the interactions of the two domains represent one of the key questions for the understanding of the function of these enzymes. The CBDs of fungal cellulases are small peptides folding into a rigid, disulfidestabilized structure that has a distinct cellulose binding face. Here we describe properties of a recombinant double CBD, constructed by fusing the CBDs of two Trichoderma reesei cellobiohydrolases via a linker peptide similar to the natural cellulase linkers. After expression in Escherichia coli, the ***protein*** was ***purified*** from the culture medium by reversed phase chromatography and the individual domains obtained by trypsin digestion. Binding of the double CBD and its single CBD components was investigated on different types of cellulose substrates as well as chitin. Under saturating conditions, nearly 20 mu-mol/g of the double CBD was bound onto microcrystalline cellulose. The double CBD exhibited much higher affinity on cellulose than either of the single CBDs, indicating an interplay between the two components. A two-step model is proposed to explain the binding behavior of the double CBD. A similar interplay between the domains in the native enzyme is suggested for its binding to cellulase.

L19 ANSWER 10 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:314711 BIOSIS
DN PREV199598329011
TI Purification of human interleukin-2 using the ***cellulose*** - ***binding*** ***domain*** of a prokaryotic cellulase.
AU Ong, Edgar; Alimonti, Judie B.; Greenwood, Jeffrey M.; Miller., Robert C., Jr.; Warren, R. Antony J.; Kilburn, Douglas G. (1)
CS (1) Dep. Microbiol. Immunol., Univ. British Columbia, 300-6174 University Blvd., Vancouver, British Columbia V6T 1Z3 Canada
SO Bioseparation, (1995) Vol. 5, No. 2, pp. 95-104.
ISSN: 0923-179X.
DT Article
LA English
AB Engineering gene fusions which introduce an affinity tag linked to the target polypeptide by a specific protease cleavage site is widely used to facilitate recombinant ***protein*** ***purification*** . A fusion ***protein*** CBD-APT-IL-2, comprised of the ***cellulose*** - ***binding*** ***domain*** (CBD) and Pro-Thr (PT) rich linker of the Cellulomonas fimi endo-beta-1,4-glucanase A (CenA) and a factor X-q cleavage sequence (IleGluGlyArg) fused to the N terminus of human interleukin-2, was produced in Escherichia coli, Streptomyces lividans and mammalian COS cells. CBD-APT-IL-2, secreted from S. lividans or COS cells or recovered from the insoluble fraction of E. coli, could be purified by adsorption on cellulose. The intact fusion protein adsorbed to cellulose was hydrolyzed in situ with factor X-a to release active interleukin-2.

L19 ANSWER 11 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1995:291432 BIOSIS
DN PREV199598305732
TI *Construction of a* ***Protein*** ***Purification*** *and Enzyme* Immobilization System by Using a ***Cellulose*** ***Binding*** ***Domain*** from Clostridium cellulovorans Cellulase.
AU Park, Jae-Seon; Shin, Hae-Sun; Doi, Roy H.
CS Univ. California, Davis, CA USA
SO Abstracts of the General Meeting of the American Society for Microbiology, (1995) Vol. 95, No. 0, pp. 372.
Meeting Info.: 95th General Meeting of the American Society for Microbiology Washington, D.C., USA May 21-25, 1995
ISSN: 1060-2011.
DT Conference
LA *English*

L19 ANSWER 12 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:272484 BIOSIS
DN PREV199598286784
TI Expression, purification, and characterization of the ***cellulose*** - ***binding*** ***domain*** of the scaffoldin subunit from the cellulosome of Clostridium thermocellum.
AU *Morag, Ely; Lapidot, Aviva; Govorko, Dmitry; Lamed, Raphael; Wilchek,* Meir; Bayer, Edward A.; Shoham, Yuval (1)
CS (1) Dep. Food Eng. Biotechnol., *Technion, Haifa* 32000 Israel
SO Applied and Environmental Microbiology, (1995) Vol. 61, No. 5, pp. 1980-1986.
ISSN: 0099-2240.
DT Article
LA English
AB The major ***cellulose*** - ***binding*** ***domain*** (CBD) from the cellulosome of *Clostridium thermocellum YS* was cloned and overexpressed in Escherichia coli. The expressed ***protein*** was ***purified*** *efficiently by a modification of a novel procedure termed* affinity digestion. The properties of the purified polypeptide were compared with those of a related CBD derived from a cellulosome-like complex of a similar (but mesophilic) clostridial species, Clostridium cellulovorans. The binding properties of the two proteins with their common substrate were found to be very similar. Despite the similarity in the amino acid sequences of the two CBDs, polyclonal antibodies raised against the CBD from C. thermocellum failed to interact with the protein from C. cellulovorans. Chemical modification of the single cysteine of the *CBD had little effect on the binding to cellulose. Biotinylation of this* cysteine allowed the efficient binding of avidin to cellulose, and the *resultant matrix is appropriate for use as a universal affinity system.*

L19 ANSWER 13 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:172236 BIOSIS
DN PREV199598186536
TI Cloning and expression in Saccharomyces cerevisiae of a Trichoderma reesei beta-mannanase gene containing a ***cellulose*** ***binding*** ***domain*** .
AU *Stalbrand, Henrik; Saloheimo, Anu; Vehmaanpera, Jari; Henrissat, Bernard;* Penttila, Merja (1)
CS (1) VTT Biotechnol. Food Res., P.O. Box 1500, FIN-02044 VTT, Espoo Finland
SO Applied and Environmental Microbiology, (1995) Vol. 61, No. 3, pp. 1090-1097.
ISSN: 0099-2240.
DT Article
LA English
AB *beta-Mannanase (endo-1,4-beta-mannanase; mannan endo-1,4-beta-*mannosidase;
EC 3.2.1.78) catalyzes endo-wise hydrolysis of the backbone of mannan and heteromannans, including hemicellulose polysaccharides, which are among the major components of plant cell walls. The gene man), which encodes beta-mannanase, of the filamentous fungus Trichoderma reesei was isolated from an expression library by using antiserum raised towards the earlier- ***purified*** beta-mannanase ***protein*** . The deduced beta-mannanase consists of 410 amino acids. On the basis of hydrophobic cluster analysis, the beta-mannanase was assigned to family 5 of glycosyl hydrolases (cellulase family A). The C terminus of the beta-mannanase has *strong amino acid sequence similarity to the cellulose binding domains of* fungal cellulases and is preceded by a serine-, threonine-, and proline-rich region. Consequently, the beta-mannanase is probably organized similarly to the T. reesei cellulases, having a catalytic core domain separated from the substrate-binding domain by an O-glycosylated linker. Active beta-mannanase was expressed and secreted by using the yeast Saccharomyces cerevisiae as the host. The results indicate that the man1 gene encodes the two beta-mannanases with different isoelectric points (pIs 4.6 and 5.4) purified earlier from T. reesei.

L19 ANSWER 14 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1994:545309 BIOSIS
DN PREV199598004857
TI Characterization and sequence analysis of a Streptomyces rochei A2 endoglucanase-encoding gene.
AU Perito, Brunella; Hanhart, Eva; Irdani, Tiziana; Iqbal, Munir; McCarthy, Alan J.; Mastromei, Giorgio
CS *Dep. Animal Biol. Genetics, via Romana 17, 50125 Florence Italy*
SO Gene (Amsterdam), (1994) Vol. 148, No. 1, pp. 119-124.
ISSN: 0378-1119.
DT Article
LA *English*
AB A 7-kb fragment of Streptomyces rochei A2 chromosomal DNA was cloned into
pAT153 and shown to confer endoglucanase (EglS) activity on Escherichia coli cells. In E. coli clones, the EglS was secreted into the periplasm. Deletion analysis revealed that an 827-bp fragment was enough for the enzymatic activity. Sequence analysis showed that the 827-bp fragment codes for the catalytic domain of the enzyme. The complete sequence of the gene (eglS) is 1149-bp long. A signal peptide, a catalytic domain and a ***cellulose*** - ***binding*** ***domain*** *were identified from* the nucleotide sequence, and the EglS found to belong to the family H of *cellulose catalytic domains. These conclusions were substantiated by* determination of the N-terminal sequence of the ***purified*** ***protein*** and zymogram analysis, which revealed protein species with a molecular mass equal to that deduced from the nt sequence analysis.

L19 ANSWER 15 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:509931 BIOSIS
DN PREV199345108556
TI *Beta-1,4-Glycanases and beta-glycosidases.*
AU Warren, R. Anthony J.
CS Dep. Microbiol. and Protein Eng., Network Cent. Excellence, Univ. British Columbia, Number 300-6174 University Blvd., Vancouver, BC V6T 1Z3 Canada
SO Current Opinion in Biotechnology, (1993) Vol. 4, No. 4, pp. 469-473.
ISSN: 0958-1669.
DT General Review
LA English

L19 ANSWER 16 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:504538 *BIOSIS*
DN PREV199396128545
TI Characterization of the ***cellulose*** - ***binding*** ***domain*** of the Clostridium cellulovorans cellulose-binding protein A.
AU Goldstein, Marc A.; Takagi, Masahiro; Hashida, Seiichi; Shoseyov, Oded; Doi, Roy H. (1); Segel, Irwin H.
CS (1) Dep. Biochem. Biophys., Univ. Calif. Davis, Davis, CA 95616 USA
SO Journal of Bacteriology, (1993) Vol. 175, No. 18, pp. 5762-5768.
ISSN: 0021-9193.
DT Article
LA *English*
AB Cellulose-binding protein A (CbpA), a component of the cellulase complex of Clostridium cellulovorans, contains a unique sequence which has been demonstrated to be a ***cellulose*** - ***binding*** ***domain*** (CBD). The DNA coding for this putative CBD was subcloned into pET-8c, an Escherichia coli expression vector. The protein produced under the direction of the recombinant plasmid, pET-CBD, had a high affinity for crystalline cellulose. Affinity- ***purified*** CBD ***protein*** was used in equilibrium binding experiments to characterize the *interaction of the protein with various polysaccharides. It was found that* the binding capacity of highly crystalline cellulose samples (e.g., cotton) was greater than that of samples of low crystallinity (e.g., fibrous cellulose). At saturating CBD concentration, about 6.4 mu-mol of protein was bound by 1 g of cotton. Under the same conditions, fibrous cellulose bound only 0.2 mu-mol of CBD per g. The measured dissociation constant was in the 1 mu-M range for all cellulose samples. The results suggest that the CBD binds specifically to crystalline cellulose. Chitin, which has a crystal structure similar to that of cellulose, also was bound *by the CBD. The presence of high levels of cellobiose or carboxymethyl* cellulose in the assay mixture had no effect on the binding of CBD protein to crystalline cellulose. This result suggests that the CBD recognition site is larger than a simple cellobiose unit or more complex than a repeating cellobiose moiety. This CBD is of particular interest because it is the first CBD from a completely sequenced nonenzymatic protein shown to be an independently functional domain.

L19 ANSWER 17 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:428946 BIOSIS
DN BA94:81071
TI CELLULOSE-BINDING DOMAINS POTENTIAL FOR PURIFICATION OF COMPLEX PROTEINS.
AU GREENWOOD J M; ONG E; GILKES N R; WARREN R A J; MILLER R C JR; KILBURN D G
CS DEP. MICROBIOL., UNIV. B.C., VANCOUVER, B.C., CAN. V6T 1Z3.
SO PROTEIN ENG, (1992) 5 (4), 361-365.
CODEN: PRENE9. ISSN: 0269-2139.
FS BA; OLD
LA *English*
AB The endoglucanase CenA and the exoglucanase Cex from Cellulomonas fimi *each contain a discrete* ***cellulose*** - ***binding*** ***domain*** (CBD), at the amino-terminus or carboxyl-terminus respectively. The gene fragment encoding the CBD can be fused to the gene of a protein of interest. Using this approach hybrid proteins can be engineered which bind reversibly to cellulose and exhibit the biological activity of the protein partner. Alkaline phosphatase (PhoA) from Escherichia coli, and a .beta.-glucosidase (Abg) from an Agrobacterium sp. are dimeric proteins. The fusion polypeptides CenA-PhoA and Abg-CBCCex are sensitive to proteolysis at the junctions between the fusion partners.

Proteolysis results in a mixture of homo- and heterodimers; these bind to cellulose if one or both of the monomers carry a CBD, e.g. CenA-PhoA CenA-PhoA and CenA-PhoA/PhoA. CBD fusion polypeptides could be used in this way to purify polypeptides which associate with the fusion partner.

L19 ANSWER 18 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:210950 BIOSIS
DN BA93:111175
TI PURIFICATION AND CHARACTERIZATION OF AN ENDOGLUCANASE FROM
STREPTOMYCES-LIVIDANS 66 AND DNA SEQUENCE OF THE GENE.
AU THEBERGE M; LACAZE P; SHARECK F; MOROSOLI R; KLUEPFEL D
CS CENT. RECHERCHE EN MICROBIOL. APPLIQUEE, INST. ARMAND-FRAPPIER, UNIV.
QUEBEC, 531 BLVD. DES PRAIRIES, LAVAL-DES-RAPIDES, QUEBEC, CANADA H7N 4Z3.
SO APPL ENVIRON MICROBIOL, (1992) 58 (3), 815-820.
CODEN: AEMIDF. ISSN: 0099-2240.
FS BA; OLD
LA English
AB The endoglucanase isolated from culture filtrates of Streptomyces lividans IAF74 was shown to have a Mr of 46,000 and a pI of 3.3. The specific enzyme activity of 539 IU/mg, determined by the reducing assay method on carboxymethyl cellulose, is among the highest reported in the literature. The cellulase showed typical endo-type activity when reacting on oligocellodextrins. Optimal enzyme activity was obtained at 50.degree. C and pH 5.5. The kinetic constants for this endoglucanase, determined with carboxymethyl cellulose as the substrate, were a Vmax of 24.9 IU/mg of enzyme and a Km of 4.2 mg/ml. Activity was found against neither methylumbelliferyl- nor p-nitrophenyl-cellobiopyranoside nor with xylan. The DNA sequence contains one possible reading frame validated by the N terminus of the mature ***purified*** ***protein*** . However, neither ATG nor GTG starting codons were identified near the ribosome-binding site. A putative TTG codon was found as a good candidate for the start codon. Comparison of the primary amino acid sequence of the endoglucanase of S. lividans revealed that the N terminus contains a bacterial ***cellulose*** - ***binding*** ***domain*** . The catalytic domain the C terminus showed similarity to endoglucanases from a Bacillus sp. Thus, the endoglucanase CelA belongs to family A of cellulases as described before (N.R. Gilkes, B. Henrissat, D.G. Kilburn, R.C. Miller, Jr., and R.A.J. Warren, Microbiol. Rev. 55:303-315, 1991).

L19 ANSWER 19 OF 28 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1989:501135 BIOSIS
DN BR37:110794
TI THE CELLULOSE-BINDING DOMAINS OF CELLULASES TOOLS FOR BIOTECHNOLOGY.
AU ONG E; GREENWOOD J M; GILKES N R; KILBURN D G; MILLER R C JR; WARREN R A J
CS DEP. MICROBIOL., UNIV. B.C., 300-6174 UNIVERSITY BLVD., VANCOUVER, B.C.
V6T 1W5, CAN.
SO Trends Biotechnol., (1989) 7 (9), 239-243.
CODEN: TRBIDM. ISSN: 0167-7799.
FS BR; OLD
LA English

L19 ANSWER 20 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1999037044 EMBASE
TI ***Purification*** of a fusion ***protein*** using the family VI ***cellulose*** - ***binding*** ***domain*** of Clostridium stercorarium XynA.
AU Sakka K.; Karita S.; Kemura T.; Ohmiya K.
CS K. Sakka, Faculty of Bioresources, Mie University, Tsu 514, Japan
SO Annals of the New York Academy of Sciences, (1998) 864/- (485-488).
Refs: 5
ISSN: 0077-8923 CODEN: ANYAA
CY United States
DT Journal; Conference Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English

L19 ANSWER 21 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1999004926 EMBASE
TI Involvement of both dockerin subdomains in assembly of the Clostridium thermocellum cellulosome.
AU Lytle B.; David Wu J.H.
CS J.H. David Wu, University of Rochester, Department of Chemical Engineering, 206 Gavett Hall, Rochester, NY 14627-0166, United States.
davidwu@che.rochester.edu
SO Journal of Bacteriology, (1998) 180/24 (6581-6585).
Refs: 26
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Clostridium thermocellum produces an extracellular cellulase complex termed the cellulosome. It consists of a scaffolding protein, CipA, containing nine cohesin domains and a ***cellulose*** - ***binding*** ***domain*** , and at least 14 different enzymatic subunits, each containing a conserved duplicated sequence, or dockerin domain. The cohesin-dockerin interaction is responsible for the assembly of the catalytic subunits into the cellulosome structure. Each duplicated sequence of the dockerin domain contains a region bearing homology to the EF-hand calcium-binding motif. Two subdomains, each containing a putative calcium-binding motif, were constructed from the dockerin domain of CelS, a major cellulosomal catalytic subunit. These subdomains, called DS1 and DS2, were cloned by PCR and expressed in Escherichia coli. The binding of DS1 and DS2 to R3, the third cohesin domain of CipA, was analyzed by nondenaturing gel electrophoresis. A stable complex was formed only when R3 was combined with both DS1 and DS2, indicating that the two halves of the dockerin domain interact with each other and such interaction is required for effective binding of the dockerin domain to the cohesin domain.

L19 ANSWER 22 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 1998307647 EMBASE
TI Determinants of recombinant production of antimicrobial cationic peptides and creation of peptide variants in bacteria.
AU Zhang L.; Falla T.; Wu M.; Fidai S.; Burian J.; Kay W.; Hancock R.E.W.
CS R.E.W. Hancock, Department Microbiology Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Canada. bob@cmdr.ubc.ca
SO Biochemical and Biophysical Research Communications, (29 Jun 1998) 247/3 (674-680).
Refs: 30
ISSN: 0006-291X CODEN: BBRCA
CY United States
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
037 Drug Literature Index
039 Pharmacy
LA English
SL English
AB Cationic peptides possessing antibacterial activity are virtually ubiquitous in nature, and offer exciting prospects as new therapeutic agents. We had previously demonstrated that such peptides could be produced by fusion protein technology in bacteria and several carrier proteins had been tested as fusion partners including glutathione-S-transferase, S. aureus protein A, IgG binding protein and P. aeruginosa outer membrane protein OprF. However these fusion partners, while successfully employed in peptide expression, were not optimized for high level production of cationic peptides. In this paper we took advantage of a small replication protein RepA from E. coli and used its truncated version to construct fusion partners. The minimal elements required for high level expression of cationic peptide were defined as a DNA sequence encoding a fusion protein comprising, from the N-terminus, a 68 amino acid carrier region, an anionic prepro domain, a single methionine and the peptide of interest. The 68 amino acid carrier region was a block of three polypeptides consisting of a truncated RepA, a synthetic ***cellulose*** ***binding*** ***domain*** and a hexa histidine domain. The improved system showed high level expression and simplified downstream purification. The active peptide could be yielded by CNBr cleavage of the fusion protein. This novel vector was used to express three classes of cationic peptides including the .alpha.-helical peptide CEMA, the looped peptide bactenecin and the extended peptide indolicidin. In addition, mutagenesis of the peptide gene to produce peptide variants of CEMA and indolicidin using the improved vector system was shown to be successful.

L19 ANSWER 23 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 97259933 EMBASE
DN 1997259933
TI Very high-level production and export in Escherichia coli of a ***cellulose*** ***binding*** ***domain*** for use in a generic secretion-affinity fusion system.
AU Hasenwinkle D.; Jervis E.; Kops O.; Liu C.; Lesnicki G.; Haynes C.A.; Kilburn D.G.
CS C.A. Haynes, Chemical Engineering, Wesbrook Building, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. israels@chml.ubc.ca
SO Biotechnology and Bioengineering, (1997) 55/6 (854-863).
Refs: 44
ISSN: 0006-3592 CODEN: BIBIAU
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB A novel expression vector pTugA, previously constructed in our laboratory, was modified to provide kanamycin resistance (pTugK) and used to direct the synthesis of polypeptides as fusions with the C- or N-terminus of a ***cellulose*** ***binding*** ***domain*** which serves as the affinity tag in a novel secretion-affinity fusion system. Fed-batch fermentation strategies were applied to production in recombinant E. coli TOPP5 of the ***cellulose*** ***binding*** ***domain*** (CBD) from the Cellulomonas fimi cellulase Cex. The pTugK expression vector, which codes for the Cex leader sequence that directs the recombinant protein to the periplasm of E. coli, was shown to remain stable at very high- cell densities. Recombinant cell densities in excess of 90 g (dry cell weight)/L were achieved using media and feed solutions optimized using a 2(n) factorial design. Optimization of inducer (isophenyl-thio-.beta.-D- galactopyranoside) concentration and the time of

induction led to soluble, fully active CBD(Cex) production levels in excess of 8 g/L.

L19 ANSWER 24 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 96293768 EMBASE
DN 1996293768
TI Cloning, sequencing, and expression of the cellulase genes of Humicola grisea var. thermoidea.
AU Takashima S.; Nakamura A.; Hidaka M.; Masaki H.; Uozumi T.
CS Department of Biotechnology, Faculty of Agriculture, University of Tokyo, Yayoi 1-1-1,Bunkyo-ku, Tokyo 113, Japan
SO Journal of Biotechnology, (1996) 50/2-3 (137-147).
ISSN: 0168-1656 CODEN: JBITD4
CY Netherlands
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB We have cloned an endoglucanase (EGI) gene and a cellobiohydrolase (CBHI)
gene of Humicola grisea var. thermoidea using a portion of the Trichoderma reesei endoglucanase I gene as a probe, and determined their nucleotide sequences. The deduced amino acid sequence of EGI was 435 amino acids in length and the coding region was interrupted by an intron. The EGI lacks a hinge region and a ***cellulose*** - ***binding*** ***domain*** . The deduced amino acid sequence of CBHI was identical to the H. grisea CBHI previously reported, with the exception of three amino acids. The H. grisea EGI and CBHI show 39.8% and 37.7% identity with the T. reesei EGI, respectively. In addition to TATA box and CAAT motifs, putative CREA binding sites were observed in the 5' upstream regions of both genes. The cloned cellulase genes were expressed in Aspergillus oryzae and the gene products were purified. The optimal temperatures of CBHI and EGI were 60.degree.C and 55-60.degree.C, respectively. The optimal pHs of these enzymes were 5.0. CBHI and EGI had distinct substrate specificities: CBHI showed high activity toward Avicel, whereas EGI showed high activity toward carboxymethyl cellulose (CMC).

L19 ANSWER 25 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 94364358 EMBASE
DN 1994364358
TI Mutation analysis of the ***cellulose*** - ***binding*** ***domain*** of the Clostridium cellulovorans cellulose-binding protein A.
AU Goldstein M.A.; Doi R.H.
CS Section of Molecular/Cell. Biology, University of California,Davis, CA 95616, United States
SO Journal of Bacteriology, (1994) 176/23 (7328-7334).
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Cellulose-binding protein A (CbpA) has been previously shown to mediate the interaction between crystalline cellulose substrates and the cellulase enzyme complex of Clostridium cellulovorans. CbpA contains a family III ***cellulose*** - ***binding*** ***domain*** (CBD) which, when expressed independently, binds specifically to crystalline cellulose. A series of N- and C-terminal deletions and a series of small internal deletions of the CBD were created to determine whether the entire region previously described as a CBD is required for the cellulose-binding function. The N- and C-terminal deletions reduced binding affinity by 10- to 100-fold. Small internal deletions of the CBD resulted in substantial reduction of CBD function. Some, but not all, point mutations throughout the sequence had significant disruptive effects on the binding ability of the CBD. Thus, mutations in any region of the CBD had effects on the binding of the fragment to cellulose. The results indicate that the entire 163-amino-acid region of the CBD is required for maximal binding to crystalline cellulose.

L19 ANSWER 26 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 94354486 EMBASE
DN 1994354486
TI Purification and processing of ***cellulose*** - ***binding*** ***domain*** -alkaline phosphatase fusion proteins.
AU Greenwood J.M.; Gilkes N.R.; Miller Jr. R.C.; Kilburn D.G.; Warren R.A.J.
CS Department Microbiology/Immunology, University of British Columbia,Vancouver, BC V6T 1Z3, Canada
SO Biotechnology and Bioengineering, (1994) 44/11 (1295-1305).
ISSN: 0006-3592 CODEN: BIBIAU
CY United States
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB Fusion of the leader peptide and the ***cellulose*** - ***binding*** ***domain*** (CBD) of endoglucanase A (CenA) from Cellulomonas fimi, with or without linker sequences, to the N-terminus of alkaline phosphatase (PhoA) from Escherichia coli leads to the accumulation of significant amounts of the CBD-PhoA fusion proteins in the supernatants of E. coli cultures. The fusion proteins can be purified from the supernatants by affinity chromatography on cellulose. The fusion proteins can be desorbed from the cellulose with water or guanidine-HCl. If the sequence IEGR is present between the CBD and PhoA, the CBD can be cleaved
from the PhoA with factor Xa. The efficiency of hydrolysis by factor Xa is strongly influenced by the amino acids on either side of the IEGR sequence. The CBD released by factor Xa is removed by adsorption to cellulose. A nonspecific protease from C. fimi, which hydrolyzes native CenA between the CBD and the catalytic domain, may be useful for removing the CBD from some fusion proteins.

L19 ANSWER 27 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 93293223 EMBASE
DN 1993293223
TI Production and properties of a factor X- ***cellulose*** - ***binding*** ***domain*** fusion protein.
AU Assouline Z.; Shen H.; Kilburn D.G.; Warren R.A.J.
CS Department of Microbiology, University of British Columbia, 300-6174 University Boulevard,Vancouver, BC V6T 1Z3, Canada
SO Protein Engineering, (1993) 6/7 (787-792).
ISSN: 0269-2139 CODEN: PRENE
CY United Kingdom
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB A fusion protein, FX-CBD(Cex), which comprises factor X with a ***cellulose*** - ***binding*** ***domain*** (CBD(Cex)) fused to its C-terminus, was produced in BHK cells. It was purified from the culture medium by affinity chromatography on cellulose. FX-CBD(Cex) could be activated to FXa-CBD(Cex) with Russell viper venom. FXa-CBD(Cex) was as active as FXa against a chromogenic substrate and against proteins containing the Ile-Glu-Gly-Arg sequence hydrolysed by FXa. FXa-CBD(Cex) retained its activity when adsorbed to cellulose.

L19 ANSWER 28 OF 28 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 89050499 EMBASE
DN 1989050499
TI Fusion to an endoglucanase allows alkaline phosphatase to bind to cellulose.
AU Greenwood J.M.; Gilkes N.R.; Kilbrun D.G.; Miller Jr. R.C.; Warren R.A.J.
CS Department of Microbiology, University of British Columbia, Vancouver, BC V6T 1W5, Canada
SO FEBS Letters, (1989) 244/1 (127-131).
ISSN: 0014-5793 CODEN: FEBLAL
CY Netherlands
DT Journal
FS 004 Microbiology
047 Virology
LA English
SL English
AB Endoglucanase CenA of Cellulomonas fimi comprises an N-terminal ***cellulose*** - ***binding*** ***domain*** and a C-terminal catalytic domain joined together by a sequence of 24 proline and threonine residues (the Pro-Thr box). The domains function independently when separated by proteolysis. TnphoA has been used to generate cenA'-'phoA fusions. CenA'-'PhoA fusion polypeptides which contain the entire ***cellulose*** - ***binding*** ***domain*** of CenA bind to cellulose, allowing their purification from periplasmic extracts in a single, facile step. This result has implications for purification or immobilisation of chimeric proteins on a cheap cellulose matrix.

=>

---Logging off of STN---

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Executing the logoff script...

=> LOG Y

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COST IN U.S. DOLLARS SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST 91.97 257.86

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) SINCE FILE TOTAL
ENTRY SESSION
CA SUBSCRIBER PRICE 0.00 -9.92

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